nature reviews clinical oncology

https://doi.org/10.1038/s41571-025-01077-z

Review article

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The tumour microenvironment in pancreatic cancer — new clinical challenges, but more opportunities

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Abstract

Patients with advanced-stage pancreatic ductal adenocarcinoma (PDAC) predominantly receive chemotherapy, and despite initial responses in some patients, most will have disease progression and often dismal outcomes. This lack of clinical effectiveness partly reflects not only cancer cell-intrinsic factors but also the presence of a tumour microenvironment (TME) that precludes access of both systemic therapies and circulating immune cells to the primary tumour, as well as supporting the growth of PDAC cells. Combined with improved preclinical models of PDAC, advances in single-cell spatial multi-omics and machine learning-based models have provided novel methods of untangling the complexities of the TME. In this Review, we focus on the desmoplastic stroma and both the intratumoural and intertumoural heterogeneity of PDAC, with an emphasis on cancer-associated fibroblasts and their surrounding immune cell niches. We describe new approaches in converting the immunologically 'cold' PDAC TME into a 'hot' TME by priming T cell activation, overcoming T cell exhaustion and unravelling myeloid cell-mediated immunosuppression. Furthermore, we explore integrated targets involving the TME, such as points of convergence among tumour, stromal and immune cell metabolism as well as oncogenic KRAS signalling. Finally, building on our experience with failed clinical trials in the past, we consider how this evolving comprehensive understanding of the TME will ensure future success in developing more effective therapies for patients with PDAC.

Sections

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Key points

- Heterogeneity within the pancreatic ductal adenocarcinoma (PDAC) tumour microenvironment (TME) is highlighted by differences in both the structure and cellular origin of fibrillar collagen present within the extracellular matrix, with distinct tumour-promoting and tumour-restraining roles.
- Given the heterogeneity and plasticity of cancer-associated fibroblasts in the PDAC TME, research efforts have focused on elucidating the specific tumour-permissive and tumour-restrictive functions of these different subpopulations and targeting them with highly specific therapeutic interventions.
- Cancer-associated fibroblast function is greatly dependent on the specific niche and cellular neighbourhood, and advances in multi-omic, spatial analysis technologies have enabled assessments of the spatial relationships and inference of cellular interactions between neoplastic and stromal components with validation in in vitro models.
- Therapeutic cancer vaccines are capable of presenting PDAC-associated antigens to the immune system to mount an antitumour effector T cell response, including by converting an immunologically 'cold' TME into a 'hot' one.
- Future research efforts should focus on reprogramming immunosuppressive myeloid cells in the immune TME to prevent T cell exhaustion and sustain effector T cell activation.
- The potential of TME remodelling to bypass dependencies on mutant KRAS supports the further exploration of strategies targeting the TME in combination with, or following KRAS inhibitors to overcome resistance to these agents.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), which accounts for the majority of pancreatic cancers, is a devastating malignancy. Since our previous Review describing the PDAC tumour microenvironment (TME) was published in 2020 (ref. 1), the 5-year overall survival (OS) of patients with PDAC in the USA and other Western countries has slowly increased to slightly above 10% (ref. 2), although this improvement can largely be attributed to the optimization of multidisciplinary care rather than to innovative therapies that have been tested in clinical trials[3]. The importance of targeting the TME in PDAC continues to be underscored; however, developing agents capable of targeting the TME is challenging owing to substantial intratumoural and intertumoural heterogeneity[4]. The complexity of the TME is further increased by the capacity for dynamic reprogramming, driven by neoplastic cells that are able to constantly adapt to changes in the TME as well as exposure to treatments[5]. The latter consideration has redirected our attention to tumour-intrinsic mechanisms of resistance and the development of molecularly targeted therapies for selected patients with PDAC[6].

Over the past 5 years, considerable progress has been made in our understanding of the PDAC TME, aided by advances in technology such as single-cell spatial multi-omics techniques combined with machine learning-based analysis for target identification[6]. These technologies have provided the precision to identify new therapeutic targets specifically within the TME of different patient subgroups and enabled us to begin elucidating the dynamic changes occurring in the TME in response to both conventional and newly emerging therapies. More encouragingly, new agents designed to target the most prevalent driver mutations in PDAC, specifically *KRAS*^{G12} mutations, have made this historically undruggable target druggable and are enabling more precise disruption of tumour-intrinsic mechanisms driving TME reprogramming[7].

Nonetheless, considerable challenges remain in translating data from single-cell and spatial analysis of the TME into effective therapeutic options for patients with PDAC. The rapid development of resistance to KRAS-targeted therapies has created a demand for other effective modulators of the TME capable of overcoming the mechanisms of acquired resistance to these agents. Ultimately, the availability of effective novel therapies for patients with PDAC will require a comprehensive understanding of the TME as well as innovations in therapeutic development strategies.

Targeting the tumour stroma

Desmoplasia, in which cancer cells activate fibroblasts to trigger fibrosis and the deposition of extracellular matrix (ECM), is a major hallmark of the PDAC TME and often comprises the bulk of the tumour[8,9]. The robust desmoplastic reaction, which creates a thick mechanical barrier that limits vascularization, drug delivery and immune infiltration, has an established role in the pathogenesis of PDAC as well as resistance to treatment[9–11]. However, the observation of more aggressive tumour growth following direct depletion of the desmoplasia via *SHH* knockout in mouse models suggests a need for a deeper understanding of this process[12]. This paradoxical finding might reflect that the dense stroma also provides a supportive network for immune cells and restricts the growth and spread of neoplastic cells[12,13]. Thus, integration of this wealth of knowledge in addition to a more contextualized understanding of the cellular interactions occurring in the TME will be necessary for the development of both targeted and multifaceted therapies involving the stroma.

Role of the extracellular matrix

The ECM of PDAC comprises a dense network of glycoproteins, proteoglycans, enzymes and secreted factors that provides physical support as well as biochemical signals for surrounding cellular components[14]. During the desmoplastic response, neoplastic and stromal cells, comprising mostly cancer-associated fibroblasts (CAFs), deposit a large amount of fibrotic ECM, particularly type I, III and IV collagens, in the TME[15] (Fig. 1). Beyond simply providing structural support, these collagens directly regulate PDAC cell proliferation, survival and migration[15]. Furthermore, varying levels of specific collagens are associated with different clinical outcomes. For example, one retrospective study found that patients with high levels of collagen I (defined as above the cohort median) had a median OS duration of 6.4 months compared with 14.6 months in those with low collagen I levels[16]. Similarly, a separate study using publicly available RNA sequencing data found that PDACs harbouring higher levels of type I, II or IV collagens, specifically including COL1A2, COL2A1 and COL4A1, had inferior OS[17].

However, a proteomic analysis of the ECM involving mouse models and samples obtained from patients with PDAC demonstrated that distinct stromal cell-derived ECM components can be positively or negatively correlated with survival outcomes[18]. By contrast, elevated levels of the remaining neoplastic cell-derived ECM proteins tended to be associated with inferior OS[18]. A follow-up study found that the

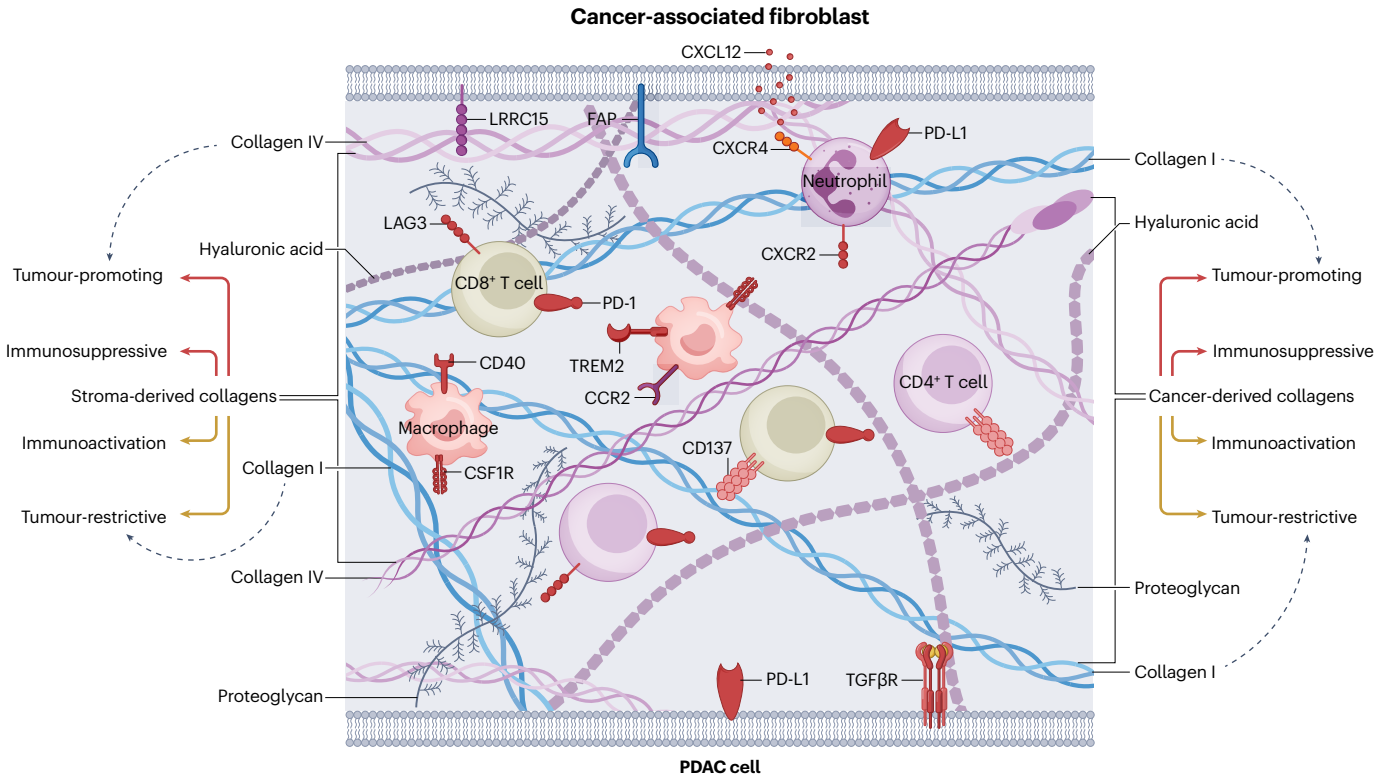

Fig. 1 | Distinct functions of the tumour-derived and stroma-derived extracellular matrix. Distinct structures within the cancer cell-derived and stromal cell-derived extracellular matrix can have tumour-promoting and tumour-restrictive roles. Relevant receptors and ligands are labelled, some of which might also be potential therapeutic targets. CCR2, C-C motif chemokine receptor 2; CSF1R, colony-stimulating factor 1 receptor; CXCL12, C-X-C motif chemokine ligand 12; CXCR2, C-X-C motif chemokine receptor 2; CXCR4, C-X-C motif chemokine receptor 4; FAP, fibroblast-activated protein; LAG3, lymphocyte activation gene 3; LRRC15, leucine-rich repeat containing 15; PDAC, pancreatic ductal adenocarcinoma; TGFβR, transforming growth factor-β receptor; TREM2, triggering receptor expressed on myeloid cells 2.

PDAC ECM is enriched with fibrillar collagens with partially uncleaved C-terminal prodomains owing to low procollagen C-proteinase activity[19]. Interestingly, only procollagen cleavage and deposition by neoplastic and not stromal cells restrained tumour growth, revealing an unexpected tumour-restraining function of neoplastic cell-derived collagen I, independent of the effects of stromal cell-derived collagens[19]. In terms of the functions of stromal cell-derived collagen I, a genetically engineered mouse model (GEMM) of spontaneous PDAC with deletion of *COL1* specifically in myofibroblasts had a TME with a reduced amount of stroma, albeit with accelerated development of pancreatic in situ neoplasms (PanINs) and invasive PDAC as well as shorter OS durations[20]. Myofibroblast-specific *COL1* deletion also led to upregulation of CXCL5 in cancer cells with subsequent increased recruitment of myeloid-derived suppressor cells (MDSCs) and a reduction in $CD8^+$ T cell infiltration[20]. Elsewhere, myofibroblastic CAFs (myCAFs)-derived collagen I has been demonstrated to restrict the growth of liver metastases by mechanically restraining tumour spread in mouse models[21]. Data from another study support differing functions of collagen I derived from neoplastic versus stromal cells[22]. Collagen I derived from human and mouse PDAC cell lines was found to be exclusively composed of abnormal collagen 1A1 homotrimers owing to epigenetic silencing of *COL1A2* via promotor hypermethylation, whereas collagens 1A1 and 1A2 were present in fibroblast-derived collagen I[22]. Data from this study demonstrate that collagen I homotrimers can induce persistent neoplastic cell growth and proliferation by binding with the discoidin domain receptor (DDR1) and α3β1 integrin on neoplastic cells[22]. Genetic deletion of *COL1A1* in cancer cells also altered the microbiome of the mouse model, resulting in increased T cell accumulation in the TME and enhanced sensitivity to anti-PD-1 antibodies[22].

Taken together, these studies demonstrate that the structure and cellular origin of fibrillar collagen within the PDAC ECM has distinct functional implications, including differentiating between tumour-promoting and tumour-suppressive effects, which might explain the failure of non-selective stroma depletion to suppress PDAC growth[23–27]. Other ECM components such as integrins[28–30] and glycoproteins[31–33] might also have pivotal roles in regulating the growth, invasion and metastasis of neoplastic cells in response to various experimental therapies (Fig. 1). For example, proteoglycans, which are abundant in the PDAC ECM, are able to create high levels of interstitial pressure in the desmoplastic stroma via interactions with hyaluronic acid[34,35]. Similar to collagens, proteoglycan structures and/or composition rather than their quantities in the ECM might have implications for PDAC development. These findings warrant a reconsideration of our approach, potentially by targeting certain forms of collagen and other ECM components in a more specific manner.

Understanding CAF heterogeneity and functionality

CAFs are a major constituent of the PDAC stroma. Fibroblasts are mesoderm-derived cells that, in the absence of malignant cells, maintain tissue homeostasis by producing ECM and regulating contractility[36]. In response to stress or tissue damage, these supportive cell types are activated to facilitate wound healing via the secretion of structural proteins and immunomodulatory signals[37]. However, in PDAC, these homeostatic functions seem to be hijacked to create a desmoplastic and immunosuppressive TME[37].

Targeting CAFs clinically has thus far proven difficult given the challenges associated with fully elucidating the complex functions of this heterogenous and dynamic cell population in patients[38] (Fig. 2). Studies over the past 5 years have utilized emerging proteomic and single-cell transcriptomic technologies to categorize CAFs into distinct subtypes[39]. These include the identification of periglandular α-smooth muscle actin (αSMA)-high myCAFs, αSMA-low and IL-6 expressing inflammatory CAFs (iCAFs) and antigen-presenting CAFs (apCAFs) that express MHC II molecules[40–42]. Data from initial studies suggest that myCAFs produce ECM and regulate tissue remodelling, whereas iCAFs primarily secrete cytokines and chemokines such as IL-6 and/or CXCL1, 2 or 12, which typically mediate immunosuppression and tumour-promoting inflammation[42]. However, the exact functions of these CAF subtypes are not fully understood. Moreover, although these subtypes have remained the most widely accepted classification, numerous studies have further defined other CAF subtypes on the basis of their transcriptional profiles and specific cell-surface markers, including senescent CAFs[43,44], Meflin$^+$ CAFs[45] and mitogen-activated protein kinase-high CAFs[46].

These studies have undoubtedly demonstrated the heterogeneity of CAFs in the PDAC TME, although categorization based entirely on their cell surface and transcriptional markers probably oversimplifies the complex nature and roles of these cells. For example, CAF populations defined by a particular cell-surface marker might not be biologically relevant if the marker does not correspond with the overriding function of that population[36]. Similarly, computationally defined CAF subgroups selected on the basis of shared transcriptomic profiles can be subject to artefacts, such as different processing techniques, and might also lack biological or clinical relevance[36]. Furthermore, categorizing CAFs into distinct groups often overlooks the existence of shared markers between different CAF subtypes that are not mutually exclusive[37]. Regardless of the granularity provided by the identification of increasing numbers of CAF subsets, an urgent need exists to obtain consensus on the classification of specific CAF subtypes, particularly in PDAC, and their associated markers to better correlate findings from different research groups[37].

A more appropriate approach to defining CAF subtype would be to define CAFs on the basis of their overriding function(s) and effects (tumour-promoting versus tumour-suppressive (Fig. 2)). For example, a CAF population regulated by transforming growth factor-β (TGFβ) signalling and characterized by cell-surface expression of leucine-rich repeat-containing 15 (LRRC15) has been associated with an unfavourable clinical response to the anti-PD-L1 antibody atezolizumab in a retrospective analysis of immune-checkpoint inhibitor (ICI)-resistant advanced-stage solid tumours[47]. A follow-up study involving a PDAC GEMM demonstrated that selective depletion of LRRC15$^+$ CAFs reduced the fibroblast content of the TME and also reprogrammed CAFs towards a 'universal fibroblast phenotype' similar to native fibroblasts in the non-malignant pancreas, in association with improved intratumoural CD8$^+$ T cell effector function and increased activity of anti-PD-L1 antibodies[48] (Fig. 3a). In another study, investigators delineated two functionally distinct populations of pancreatic fibroblasts defined by CD105 expression. Co-transplantation of CD105$^+$ or CD105$^-$ fibroblasts alongside PDAC cells in various mouse models demonstrated that CD105$^+$ fibroblasts permit tumour growth, in contrast to the tumour-restraining effects of CD105$^-$ fibroblasts, which are also dependent on functional adaptive immunity[49]. Interestingly, although CD105$^-$ CAFs share their mesothelial origin and overlap transcriptionally with apCAFs (Fig. 2), a separate study demonstrates that the latter can directly engage naive CD4$^+$ T cells, including inducing their differentiation into regulatory T (T_{reg}) cells[50]. Further research is needed to explore the multifunctionality of apCAFs[50].

Research interest exists in the roles of fibroblast-activated protein-positive (FAP$^+$) and αSMA$^+$ CAFs (Fig. 2). For example, investigators demonstrated that genetic depletion of FAP$^+$ CAFs results in improved survival in mouse models of PDAC, probably owing to the ability of FAP to promote ECM degradation, whereas depletion of αSMA$^+$ CAFs results in decreased survival. These findings were attributed to differential regulation of T_{reg} cell and effector T cell infiltration[51]. Interestingly, data from this study also demonstrate that IL-6 produced by αSMA$^+$ CAFs does not promote PDAC progression, but rather drives resistance only to gemcitabine[51]. Regardless, although αSMA$^+$ CAFs can be tumour-restrictive, particularly via the secretion of type I collagen, data from other studies provide evidence of tumour-promoting effects of this CAF phenotype. For example, several studies have reported correlations between αSMA expression in the stromal compartment and stromal activity in aggressively dividing PDACs as well as with an unfavourable prognosis[52–54]. Elsewhere, investigators identified a tumour-restrictive role of meflin$^+$ αSMA-low CAFs by demonstrating the ability of meflin to inhibit αSMA expression specifically in CAFs and alter the collagen configuration of the TME[45]. αSMA$^+$ myCAFs are also functionally heterogenous. For example, the balance between tumour-restrictive collagen I and tumour-promoting hyaluronan production in the same group of myCAFs has been shown to determine the overall functional roles of this subtype (tumour-promoting or tumour-suppressive)[21] (Fig. 3a). Besides myCAFs, the majority of CAF subsets also have some level of αSMA expression[55]. These studies underscore the complexity of CAF phenotypes and suggest that approaches targeting a single specific CAF marker such as FAP are anticipated to be ineffective. Instead, research in this field should focus on targeting the main functions of CAF subtypes in relation to neoplastic cells and other stromal cells.

A major component of research efforts to delineate the tumour-permissive versus tumour-restrictive behaviours of CAFs involves a thorough interrogation of the complex interactions between these cells and other TME components including both direct cell–cell interactions and paracrine signalling (Fig. 3b). For example, tumour cell-derived TGFβ induces the activation of EGFR–HER2 signalling in myCAFs via an autocrine process mediated by amphiregulin. These EGFR-activated myCAFs can then promote the development of metastases in mouse models of PDAC[56]. Elsewhere, oncogenic KRAS signalling, arising from oncogenic *KRAS* mutations in epithelial cells, was found to activate fibroblast autocrine signalling and drive IL-33 expression in CAFs[57]. Compartment-specific deletion of *IL33* in the stroma reprogrammed the secretome of ST2 (also known as IL-1 receptor-like 1)-positive cells and led to increased expression of the EGFR ligand amphiregulin in group 2 innate lymphoid cells and T_{reg} cells[57]. This altered secretome can subsequently alter CAF phenotypes, inducing a shift from an immunosuppressive secretory (iCAF^{high}) phenotype to a myCAF^{high}

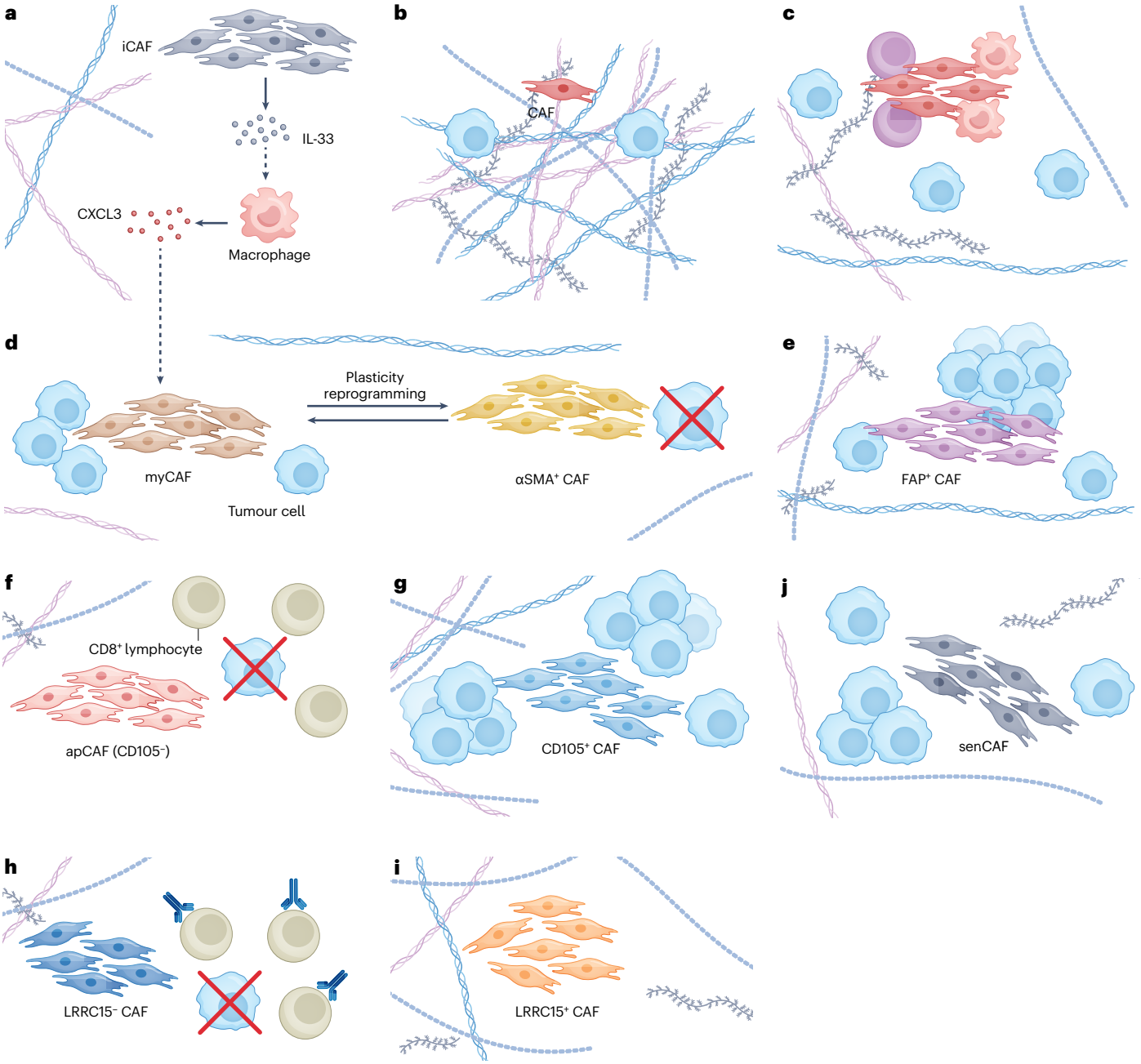

Fig. 2 | Intratumoural and intertumoural heterogeneity in the pancreatic ductal adenocarcinoma tumour microenvironment. Advances in spatial and single-cell multi-omic technologies have revealed the intratumoural and intertumoural heterogeneity of the pancreatic ductal adenocarcinoma (PDAC) tumour microenvironment (TME). Here, we provide representative sub-TMEs characterized by distinct cancer-associated fibroblasts (CAFs), extracellular matrix and immune cells. **a**, A sub-TME with inflammatory CAFs (iCAFs), which secrete pro-inflammatory cytokines, such as IL-33. **b**, A desert sub-TME, characterized by a dense collagenous stroma that impairs access of both immune cells and systemically administered therapies. **c**, A reactive sub-TME, featuring abundant immune cell infiltration, enrichment with CAFs and a limited stroma. **d**, α-Smooth muscle actin-positive myofibroblastic CAFs (αSMA$^+$ myCAFs), which are generally tumour-restrictive, but are able to transition to other less tumour-restrictive phenotypes. **e**, Fibroblast-activated protein-positive (FAP$^+$) CAFs have a tumour-promoting phenotype, partly owing to the ability of FAP to promote extracellular matrix degradation. **f**, CD105$^-$ antigen-presenting CAFs (apCAFs), associated with adaptive immunity, are tumour-restrictive. **g**, CD105$^+$ CAFs, associated with immunosuppression, are tumour-promoting. **h**, CAFs with leucine-rich repeat containing 15 (LRRC15) depletion sensitize cancer cells to immune-checkpoint inhibitors. **i**, LRRC15$^+$ CAFs are immunosuppressive owing to potentiation of resistance to immune-checkpoint inhibitors. **j**, Senescent CAFs (senCAFs), mediating immunosuppression, are tumour-promoting. CXCL3, C-X-C motif chemokine ligand 3.

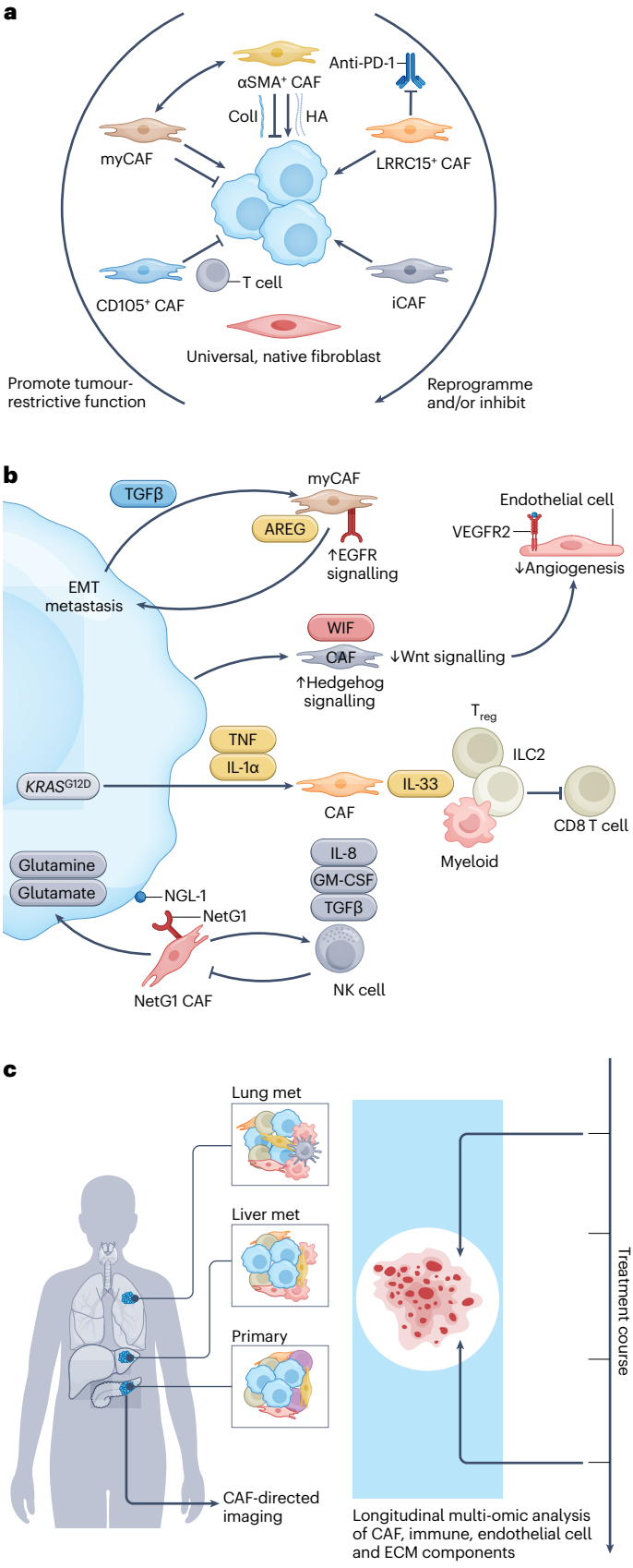

Fig. 3 | The landscape of cancer-associated fibroblast research in pancreatic ductal adenocarcinoma. a, Cancer-associated fibroblasts (CAFs) are a highly phenotypically heterogeneous population within the pancreatic ductal adenocarcinoma (PDAC) tumour microenvironment (TME). Research has largely focused on identifying the tumour-restraining and tumour-promoting functions of these cells. Inflammatory CAFs (iCAFs) and leucine-rich repeat containing protein 15-positive (LRRC15$^+$) CAFs have been shown to have a tumour-promoting phenotype, owing to the ability to limit CD8$^+$ T cell infiltration and thus confer resistance to immune-checkpoint inhibitors. CD105$^-$ CAFs are able to restrain tumour growth in an adaptive-immunity-dependent manner. α-Smooth muscle actin-positive (αSMA$^+$) CAFs and myofibroblastic CAFs (myCAFs) can have both tumour-restrictive and tumour-promoting functions, highlighting the heterogeneity in CAF functionality. These studies highlight the potential of interventions designed to reprogramme CAFs to a native universal fibroblast phenotype, thus inhibiting the tumour-permissive functions and promoting the tumour-restrictive functions of these cells. **b**, Substantial cellular crosstalk can occur between CAFs, cancer cells and other stromal cells and defines the major functions of CAFs. Upregulation of epidermal growth factor receptor (EGFR) signalling in myCAFs, owing to cancer cell–CAF crosstalk, results in the promotion of cancer cell epithelial–mesenchymal transition (EMT) and metastatic dissemination. Cancer cells are also able to upregulate hedgehog (HH) signalling in CAFs, leading to subsequent downregulation of Wnt signalling and suppression of vascular endothelial growth factor receptor 2 (VEGFR2)-dependent angiogenesis by endothelial cells. *KRAS*-mutant cancer cells can reprogramme CAFs to drive stromal cell activation and release of IL-33. Stromal cell-derived IL-33 can then promote an immunosuppressive TME by engaging ST2$^+$ cells such as type 2 innate lymphoid (ILC2) and regulatory T (T_{reg}) cells. Netrin G1 (NetG1) on CAFs engages with Netrin G1 ligand (NGL-1) on cancer cells and can promote tumour progression by supplying neoplastic cells with glutamate, glutamine and other nutrients. NetG1$^+$ CAFs can also inhibit natural killer (NK) cell-mediated cytotoxicity. Investigating such crosstalk is expected to provide a better understanding of CAF biology as well as potential therapeutic targets. **c**, Future directions in studying CAFs include characterizing CAF phenotypes and function in metastatic lesions compared with the primary tumour. Research from the past 5 years has highlighted the potential of utilizing CAF-directed imaging to select patients who might respond to CAF-directed therapy and for non-invasive treatment monitoring. Furthermore, an unmet need exists to longitudinally assess changes in CAF phenotypes, ECM components and other stromal cells in response to CAF-directed therapy and provide insights into how treatment modulates CAF functions and potential combination therapy regimens. AREG, amphiregulin; ColI, collagen I; GM-CSF, granulocyte–macrophage colony-stimulating factor; HA, hyaluronic acid; met, metastasis; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; WIF, Wnt inhibitory factor.

phenotype (Fig. 2). Stromal inactivation of IL-33 also reprogrammed myeloid cells within the TME with upregulation of pro-inflammatory chemokines such as CXCL9 and CCL2 and facilitated increased recruitment of CD8$^+$ T cells[57]. However, the functions of stromal IL-33 should be differentiated from epithelial cell-derived IL-33, which has been shown to drive epigenetic reprogramming of tumorigenesis[58], recruit tumour-promoting T_H2 cells and group 2 innate lymphoid[59] cells and drive the formation of intratumoural tertiary lymphoid structures[60]. Netrin G1 expression in CAFs can also support the viability of PDAC cells by regulating glutamate and/or glutamine metabolism and inhibiting the cytotoxic activity of natural killer (NK) cells[61]. Crucially, ablation of *NTNG1* did not affect the myofibroblastic features of CAFs or their ability to generate an abundant ECM[61]. Together, these studies highlight the mechanisms underlying some of the specific tumour-promoting effects of CAFs that could potentially be targeted while selectively maintaining the antitumour homeostatic properties of these cells.

Despite the aforementioned findings, functional interrogation of CAFs has been limited by the lack of disease models that faithfully recapitulate the characteristics of CAFs in patients, which are highly dependent on direct interactions with the ECM[37]. Thus, three-dimensional (3D) culture systems that include ECM, CAFs and other PDAC TME components provide a valuable opportunity to characterize the molecular mechanisms underlying cell–cell interactions and informing drug discovery efforts[61–67]. The currently available 3D culture systems such as organoid cultures have improved our modelling of CAFs within the TME, although these methods remain incapable of fully capturing other key elements that contribute to disease progression and treatment resistance, including immune cells and ECM. Furthermore, the extent of tumour cell and stromal heterogeneity in patients exemplifies the impossibility of accurately deciphering the crosstalk between stromal and tumour cells in a 3D culture system. Developments such as the availability of tumour explants, which enable short-term ex vivo culture of slices of human or mouse PDAC, offer a model that maintains both the pathological architecture and cellular heterogeneity of the PDAC TME[68,69]. Utilization of tumour explants followed by a single-cell regulatory network analysis uncovered a cascade of paracrine signalling that promotes hedgehog signalling in CAFs, with subsequent inhibition of non-canonical Wnt signalling in both CAFs and malignant epithelial cells as well as suppression of the extent of vascular endothelial growth factor receptor 2-dependent endothelial cell hypersprouting[70] (Fig. 3b).

Taken together, these studies suggest that a more viable approach might be to reprogramme and subsequently normalize the functions of CAFs towards a phenotype resembling a more baseline fibroblast state, a more tumour-restraining state or a less tumour-promoting state, rather than eliminating CAFs entirely[71]. As such, the effects of inducing CAF quiescence or inactivation have been explored, with preclinical data demonstrating that the vitamin D receptor ligand calipotriol[72] or all-*trans* retinoic acid[73] can induce quiescence in pancreatic stellate cells (PSCs), suppress tumour cell proliferation and increase sensitivity to chemotherapy. These preclinical data support the potential of inducing PSC quiescence in patients, although clinical feasibility might be limited in the light of the observation that PSCs give rise to a numerically minor subset of CAFs in most PDACs[74]. Furthermore, a phase II trial testing the vitamin D analogue paricalcitol plus gemcitabine and nab-paclitaxel in patients with metastatic PDAC (NCT03520790) was terminated owing to futility. However, another phase II trial testing the combination of nivolumab, nab-paclitaxel, cisplatin, gemcitabine and paricalcitol in previously untreated patients with metastatic PDAC demonstrated a promising response rate[75]. The efficacy of paricalcitol remains to be substantiated in a phase III trial. Despite this promising preliminary data, most of the challenges associated with targeting CAFs continue to exist owing to a lack of a clear distinction between tumour-promoting and tumour-restraining CAF subtypes, which also vary across different studies, probably owing to intertumoural heterogeneity.

CAFs are likely to be dynamically programmed and reprogrammed by neoplastic cells at different stages of PDAC development and progression, including in response to various treatments[76–79]. This plasticity exemplifies the limitations of our current findings, which are largely based on snapshots of the PDAC TME obtained at certain time points rather than across the entire timeline of PDAC development. Future studies attempting to determine CAF function and identify relevant targets should take into account the temporal evolution of CAFs. For example, investigators compared the matrisomes of mouse models of PDAC with varying levels of metastatic dissemination. This study found enrichment of nidogen 2 in models with the largest metastatic burden at the intermediate stages of tumour development, suggesting that nidogen 2 is a potential therapeutic target enabling the modulation of CAF function and also indicating the importance of targeting CAFs in an appropriate temporal setting[80].

Most studies investigating CAF functionality involve GEMMs with TMEs that are generally more homogeneous than those of patients with PDAC. Moreover, although genetic ablation can offer insights into the functions of a target gene, permanent effects of the deletion on the entire TME should not be equated with those of transient therapeutic interventions. Furthermore, given the context-dependent and dynamic nature of CAF functionalities, effects of genetic ablation can be confounded by the specific timing, with differing effects on CAF development versus CAFs that are already present in established tumours. These limitations must be taken into account when interpreting and attempting to translate the findings of preclinical studies involving GEMMs into clinically effective therapies against any potential targets.

Spatial characterization of stromal heterogeneity

The function of CAFs is highly dependent on the specific cellular neighbourhood or niche in which the CAFs exist, with advances in spatial multi-omic analysis technologies enabling these relationships to be investigated[71]. For example, categorization of the TMEs of samples obtained from 210 patients with advanced-stage PDAC revealed two distinct 'sub-TME' phenotypes and a third intermediate sub-TME phenotype, all rooted in fibroblast plasticity[81]. Areas of the reactive sub-TME phenotype are enriched with functionally coordinated CAFs plus an abundance of immune cells and are characterized by an aggressive basal-like tumour phenotype (Fig. 2). By contrast, the 'deserted' sub-TME, which is ECM-rich and features fewer activated fibroblasts and sparsely distributed clusters of immune cells, is associated with a chemoprotective phenotype. Not surprisingly, this study found that sub-TME phenotypes are able to shift following chemotherapy[81], specifically from having a reactive or intermediate sub-TME as the dominant phenotype to a deserted-dominant phenotype in most of the samples that were examined. These sub-TMEs often occur within the same tumour, underscoring the intratumoural heterogeneity of these phenotypes. A deserted-dominant TME is independently associated with a poor prognosis. These intratumorally heterogeneous sub-TMEs are likely to complicate assessments of prognosis and sensitivity versus resistance to chemotherapy. These findings also demonstrate that the PDAC TME probably cannot be assessed adequately without spatial multi-omics, thus precluding selection of the most effective therapies in current clinical practice.

In another study, investigators used co-detection by indexing (CODEX) multiplex immunostaining technology to spatially profile 78 PDAC resection specimens and identified several spatially defined intracellular relationships driven by highly activated and immunomodulatory cancer cells and mature and/or activated B lymphocytes[55]. Interestingly, the prognostic values of these interactions were primarily driven by cellular subpopulations located within close proximity of each other, revealing functional distinctions among specific niches within the TME. This study also integrated spatial proteomics, matrix ultrastructure and clinical metadata to generate spatial signatures predictive of survival in patients with PDAC using a machine learning model[55].

Various other spatial transcriptomic studies have mapped the spatial relationships between different cellular components of the

PDAC TME. Two studies utilized spatial technology in parallel with single-nuclear whole-transcriptome analysis to identify three multicellular communities with distinct malignant, stromal and immune features[82,83]. How these categories correlate with the three previously described sub-TMEs is unknown, although such comparisons might provide interesting conclusions. These two studies identified substantial changes in ligand–receptor interactions between CAFs and malignant cells within these communities following chemotherapy and provided further evidence supporting the computationally predicted, post-treatment enrichment of spatially defined IL-6 signalling as a mechanism of chemoresistance[82,83].

Spatial assessments of the TME have been further augmented by 3D reconstruction using spatial transcriptomics data[84,85]. For example, the Human Tumour Atlas Network (HTAN) group published a 3D reconstruction of a subcohort of 23 PDAC samples that were investigated using Visium spatial transcriptomic analysis, single-nuclear RNA sequencing and CODEX multiplex immunostaining[86]. This integrated multi-omic analysis enabled the organization of the TME into 'tumour microregions' with distinct cancer cell clusters separated by stromal components. Uniquely, this study also described microregions with similar genetic alterations, which could be further grouped into spatial subclones. This study also revealed that genetic alterations seem to be a driver of the transcriptional variations that define specific microregions and that exposure to the TME further drives heterogeneity within the microregions. This study also found distinct transcriptional patterns associated with cancer cell depth from the microregion edge and identified a specific pattern of gene expression enrichment in cancer cells located adjacent to immune cells of the TME in comparison to those located in the tumour centre. Moreover, perturbation gene-set overlap analysis demonstrated that the composition and distribution of spatial subclones in multiple solid tumours, including PDAC, resulted in varying responses to identical treatments[86]. These 3D reconstructions have added to our understanding of neoplastic cell evolution and plasticity in addition to interactions with and regional TME variations within the 3D microenvironment.

Together, these spatial and multi-omics technologies have enabled investigators to identify changes in all cell types in response to therapy and thus provide a more comprehensive understanding of changes in the entire TME, including the ECM, immune cells, cancer cells and CAFs. These new technologies have enabled dissection of the TME at a much higher resolution than was previously possible, although the findings of neighbourhood characterization of TME niches vary substantially between studies. Such variations could reflect intertumoural heterogeneity as well as the differences in technical and computational approaches. Standardizing the technical and computational approaches and establishing a human PDAC network that registers specimens or data from different cohorts might overcome the challenges associated with translating the findings of single-cell, spatial analyses and 3D reconstructions into mechanistically validated novel interventions that can improve the outcomes of patients with PDAC.

Future directions for clinical strategies targeting CAFs

As we move forward in translating preclinical findings on the roles and functions of CAFs into clinical settings, opportunities are emerging to build on past trials, including a more nuanced, targeted approach to patient selection as well as combining in-depth investigations of post-treatment samples to elucidate the effects of treatment (Fig. 3c). Specifically, substantial interest has emerged in using advanced imaging methods to identify patients with high levels of CAF activation who might respond to CAF-targeting therapies. For example, several quinoline-based FAP-targeting PET tracers have demonstrated an ability to selectively label CAFs and thus enable direct visualization of CAF density and activation[87]. This approach remains limited by the inability to differentiate between different CAF subtypes and phenotypes (for example, myCAFs versus iCAFs), although such imaging can still provide a straightforward baseline readout of CAF activity that enables gross changes following treatment to be identified. Advances in technology are expected to enable further, more precise assessments of CAF plasticity in patients, including in response to specific interventions. Longitudinal assessments of CAF dynamics will provide further insights on the optimal timing and combination of therapy, including the possibility of combining therapies targeting the immunosuppressive functions of iCAFs and the metastasis-promoting effects of myCAFs.

Characterizing changes in other TME components beyond CAFs will be a crucial step in future clinical trials. For example, particular collagens are associated with different outcomes in patients with PDAC; therefore, characterization of different collagen components in the ECM would enable researchers to assess post-treatment changes in the balance between the tumour-promoting versus tumour-restraining effects of CAFs. Other cells, such as MDSCs and endothelial cells, whose recruitment and activation are highly dependent on CAFs should also be characterized, ideally using high-resolution multi-omics in combination with ECM imaging[88]. Finally, future studies should examine CAF heterogeneity across both the primary tumour and different metastatic lesions (Fig. 3c).

Targeting immune cells

ICIs, such as anti-PD-L1 and anti-CTLA4 antibodies, have revolutionized the treatment of many solid tumours; however, these agents are largely ineffective in patients with PDAC. Specifically, microsatellite stable PDAC (comprising ~99% of all PDACs) is characterized by an immunologically 'cold' TME that limits the efficacy of ICIs owing to a lack of infiltration by effector T cells capable of recognizing antigens presented by cancer cells. This lack of T cell infiltration is supported by the dense stroma seen in most PDACs.

Therapeutic vaccines

Priming TAA-specific effector cells. Most PDACs have a low tumour mutational burden, which suggests limited numbers of possible antigens that could be recognized by tumour-reactive effector T cells, in contrast to highly immunogenic tumours such as melanoma. As such, attempts have been made to prime the immune system and mount an effective antitumour immune response by treating patients with a cancer vaccine. Our group has tested this approach using an allogeneic, irradiated, granulocyte–macrophage colony-stimulating factor (GM-CSF)-expressing whole cancer cell vaccine (GVAX)[89,90]. Tumour-associated antigens (TAAs) upregulated in two PDAC cell lines that form the GVAX, including mesothelin, PMSA, MUC1, WT1 and annexin A2, have been characterized and were found to be expressed in the majority of PDACs[91,92]. On the basis of this observation, GVAX would be anticipated to induce peripheral T cell responses in most patients[93–96]. When patients received GVAX in the neoadjuvant setting, intratumoural tertiary lymphoid aggregates (TLAs), composed of organized T cell and B cell zones, could be detected in 33 of 39 resection specimens[89,97]. In a follow-up study comparing post-treatment surgically resected tumours with matched pretreatment biopsy samples, we also identified immune cell infiltration, including $CD8^+$

effector T cells, in areas of the tumour outside the TLAs following administration of GVAX[98].

We subsequently observed the induction of PD-1$^+$ T cells and PD-L1$^+$ myeloid cells in the post-vaccination tumours. Together with preclinical data[99], these results provided the rationale for testing the combination of GVAX and the anti-PD-1 antibody nivolumab in the same neoadjuvant clinical trial platform (Fig. 4). Although adding nivolumab effectively reduced the numbers of intratumour PD-1$^+$CD4$^+$ T cells and PD-1$^+$CD8$^+$ T cells, minimal antitumour activity was observed[98]. A possible explanation for this discrepancy is that GVAX induces an insufficient number of tumour-reactive T cells. Therefore, other types of cancer vaccine, such as peptide vaccines that deliver specific tumour antigens, might be more immunogenic. Phase I/II trials have demonstrated the safety of such vaccines as well as the ability to elicit TAA-specific immune responses against WT1 or MUC1 (refs. 100–104). Data from a phase I trial published in 2018 demonstrate that 7 of 34 patients with resectable PDAC had cytotoxic T cells specific for WT1 following vaccination with dendritic cells (DCs) pulsed with both HLA I-restricted and HLA II-restricted WT1 peptides[103]. Nonetheless, the efficacy of TAA-specific peptide vaccines has not yet been substantiated beyond these early-phase trials. TAA-specific vaccines are also likely vulnerable to immune escape, which might limit durable efficacy.

T cells are not the only effectors that can potentially be activated by therapeutic vaccines. We developed a spatial multi-omics atlas of PDAC using data from the aforementioned neoadjuvant immunotherapy clinical trials[89,97,98] and demonstrated that PDACs associated with longer survival durations have TLAs that propagate plasma cells into malignant niches, implying a role for humoral immunity[105]. Our analysis also offered insights into stromal remodelling and TME priming by humoral immune effector cells in tumours obtained from patients with a response to TAA-based vaccine therapy[105].

Priming neoantigen-specific T cells. The limitations of TAA-based vaccines have led to considerable research interest in tumour neoantigens. Neoantigens are formed through various mutational events, including small insertions or deletions (indels), gene fusions and point mutations. Contrasting with TAAs, neoantigens are exclusive to cancer cells, thus circumventing central T cell tolerance when eliciting an

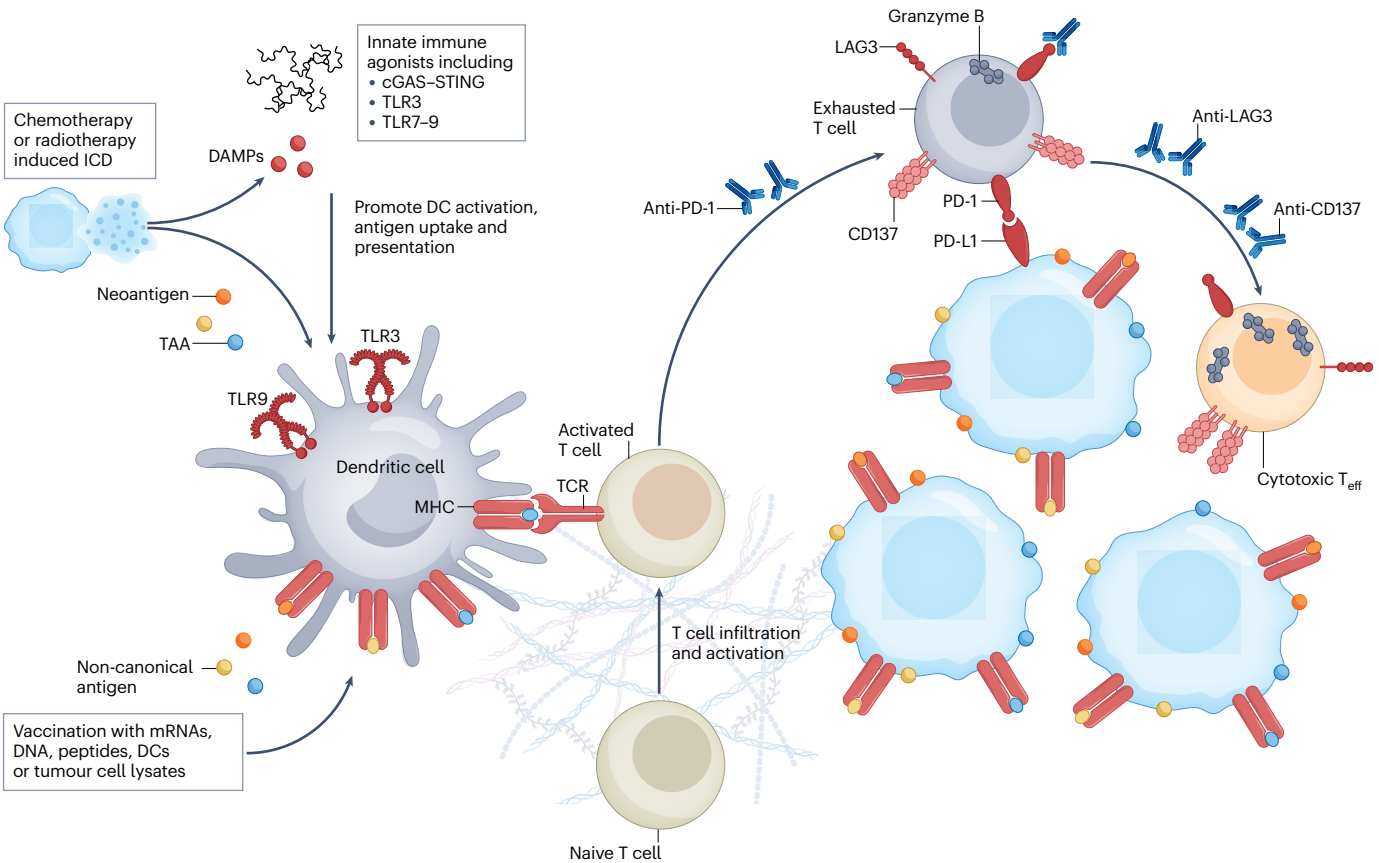

Fig. 4 | Priming the tumour microenvironment with activated T cells. T cell-mediated antitumour immunity is reliant on antigen presentation by dendritic cells (DCs) and subsequent activation and expansion of T cells. Exogenous therapeutic vaccines are able to deliver various tumour antigens, including neoantigens, tumour-associated antigens (TAAs) and non-canonical antigens, to lymphoid organs for uptake by DCs followed by T cell priming. Chemotherapy and radiotherapy can also induce immunogenic cell death (ICD), leading to the release of tumour antigens for presentation. Release of damaged-associated molecular patterns (DAMPs) following cancer cell death or additional innate immune agonists can further promote antigen uptake and presentation by DCs. However, sustained activation of T cells can lead to exhaustion, necessitating the addition of immune-checkpoint inhibitors or immune agonists to enhance the cytotoxicity of effector T cells (T_{eff}) within the tumour microenvironment (TME). cGAS–STING, cyclic guanosine monophosphate-adenosine monophosphate synthase–stimulator of interferon genes; LAG3, lymphocyte activation gene 3; TCR, T cell receptor; TLR, Toll-like receptor.

immune response[106,107] (Fig. 4). Neoantigens are typically identified using comparisons of whole-exome/genome sequencing data from tumour and non-malignant tissue samples to identify mutations[108]. These mutations are then fed into various computational pipelines designed to predict epitope presentation by MHCs, epitope–MHC stability and T cell receptor (TCR) recognition[109,110]. However, given that most neoantigens arise from individual mutations and are thus not shared across patients, a personalized approach will be required to target most neoantigens.

PDACs harbouring both the highest numbers of neoantigens and infiltrating $CD8^+$ T cells (but not either of these characteristics alone) have comparatively longer survival durations, although the immunogenicity of the neoantigens has also been identified as an important characteristic[111]. These observations led to a phase I trial testing autogene cevumeran, a personalized mRNA neoantigen vaccine comprising up to 20 MHC I-restricted or MHC II-restricted neoepitopes, in combination with a single priming dose of the anti-PD-L1 antibody atezolizumab plus modified folinic acid, fluorouracil, irinotecan and oxaliplatin (mFOLFIRINOX) as adjuvant therapy for patients with resected PDAC[112]. The vaccine was shown to be safe, and this showed increased levels of neoantigen-specific T cells in half of all patients, with vaccine-expanded T cells comprising up to 10% of all peripheral T cells in some patients. Patients with vaccine-expanded T cells and durable T cell responses had significantly improved recurrence-free survival compared with those without vaccine-expanded T cells (not reached versus 13.4 months; $P = 0.003$)[112,113]. A follow-up phase II trial testing this approach versus adjuvant mFOLFIRINOX alone in patients with resected PDAC is recruiting patients (NCT05968326).

Most neoantigens arise from individual somatic mutations, thereby necessitating a personalized vaccination approach. However, mutated *KRAS* provides an exception that has attracted considerable research interest as a shared neoantigen[114] (Fig. 4). Approximately 90% of PDACs harbour an oncogenic *KRAS* mutation, predominantly at codon 12. Furthermore, several studies have identified natural reservoirs of mutant *KRAS*-specific $CD4^+$ and $CD8^+$ T cells in patients with epithelial tumours, including PDAC[115–118]. Early attempts to vaccinate against mutant KRAS failed to demonstrate reproducible clinical benefit, although vaccine-induced T cell responses against cell lines harbouring mutant KRAS were observed[119–122]. Furthermore, the availability of systematic target discovery and validation pipelines utilizing multi-omics approaches has improved the identification of immunogenic mutant KRAS epitopes[121,122].

Several early-phase trials testing various KRAS-targeting cancer vaccines including a DC vaccine, a synthetic peptide vaccine and a small interfering RNA targeting mutant KRAS in patients with PDAC are ongoing or have provided evidence of immune responses to mutant KRAS[123–125]. Other approaches include ELI-002 2P, a three-component, lymph-node-targeted vaccine comprising long peptides derived from KRAS-G12D and KRAS-G12R, as well as the Toll-like receptor 9 (TLR9) agonist cytidine-phospho-guanosine (CpG)-7909, all with amphiphile modifications for optimal uptake[126]. A total of 25 patients (20 with resected PDAC who had detectable circulating tumour (ct)DNA without detectable cancer on imaging) received ELI-002 2P in the phase I AMPLIFY-201 trial, which demonstrated the ability to induce robust $CD8^+$ and $CD4^+$ T cell responses. Post-vaccination clearance of ctDNA was observed in three patients[126]. Our group developed a phase I trial evaluating a pooled synthetic long-peptide vaccine against KRAS-G12D, KRAS-G12R, KRAS-G12V, KRAS-G12A, KRAS-G12C and KRAS-G13D, which was administered in combination with ipilimumab and nivolumab in patients with resected PDAC[127]. Interim results (based on systemic interferon-γ (IFNγ), IL-2 and tumour necrosis factor (TNF) levels) indicate a polyfunctional T cell response in 8 of 11 patients[127]. Additionally, the therapeutic potential of targeting mutant KRAS using TCR-modified T cells is supported by a case report describing a patient with PDAC who had regression of visceral metastases following adoptive transfer of autologous T cells engineered to express two allogeneic HLA-C*08:02–restricted TCRs targeting $KRAS^{G12D}$ (ref. 128).

Whether effector T cells induced by systemic or intradermal administration of neoantigen-specific vaccines are able to traffic to the TME, as well as their functional status in patients, remains uncertain. Therefore, a need exists to track these neoantigen-specific T cells both temporally and spatially in patients following vaccination, or other approaches such as adoptive T cell transfer, which could be achieved through advances in single-cell and spatial omics technologies. Understanding whether and/or how neoantigen-specific T cells traffic to the TME would be important in expanding the clinical utility of neoantigen-based vaccines beyond minimal residual diseases[112,113].

Identifying natural non-canonical neoantigens. A neoepitope derived from a canonical neoantigen that induces a robust T cell response is only predicted in half of all PDACs, with further limitations arising from the quantity and quality of tumour specimens for DNA sequencing. Mutant KRAS is a commonly occurring neoantigen, although a strong T cell response seems to be restricted to patients with certain rare *HLA* types[128]. Neoantigens capable of illiciting a T cell response were often derived from mutations deemed to be passengers by conventional criteria, as opposed to oncogenic driver mutations[112,113]. Selecting neoantigens involves in silico prediction of the most putatively immunogenic candidates and not the identification of naturally presented antigens. Therefore, research interest has been directed towards the discovery of naturally presented T cell epitopes and non-canonical antigens across different cancer types as well as their therapeutic potential[129]. Non-canonical neoantigens are thought to reflect aberrant transcription and/or translation of presumed non-coding regions, including introns, untranslated regions of mRNAs and long non-coding RNAs or other RNAs arising from RNA editing and/or translational errors[130]. The integration of next-generation sequencing, ribosome profiling and mass spectrometry has led to a surge in the identification of such naturally presented non-canonical neoantigens over the past few years[131–133]. Non-canonical neoantigens are not subject to central tolerance as well as being more common and often shared across patients with the same primary tumour histology, potentially offering a more accessible source of tumour-specific antigens for the development of novel cancer vaccines or adoptive T cell therapies designed to promote antitumour immunity[134] (Fig. 4).

Data from numerous studies suggest that translation of nucleotide sequences from these non-coding regions can generate MHC-binding peptides and that these HLA-restricted peptides are potentially immunogenic[135–139]. In one study, investigators used a proteogenomics approach to identify 40 tumour-specific antigens from two mouse cancer cell lines and samples of 7 primary tumours from patients, of which the majority (around 90%) were presumed to originate from non-coding regions[140]. Vaccination with DCs pulsed with two non-canonical peptides provided protection against cancer cell engraftment in mouse models[140]. Leveraging 12 PDAC patient-derived organoids, a novel proteogenomics pipeline as well as high-depth immunopeptidomics, researchers identified >500 non-canonical cancer cell-specific MHC I-associated peptides (ncMAPs) specific to

cancer cells, with many being shared among different patients[141]. The proportion of ncMAPs with immunogenic potential was also substantially higher than that of canonical neoantigens or TAAs as determined by a T cell priming and expansion assay[141].

More recently, our group identified MHC I-binding variant peptides derived from erroneously translated canonical proteins, which had single amino acid substitutions[142]. These amino acid substitutions were not attributed to mutations or RNA editing, but rather seemed to result from translation errors[142]. The variant peptides were predominantly found in tumour tissues and were shared across multiple PDACs[142]. Importantly, several of these variant peptides were more immunogenic than their wild-type counterparts[142]. Taken together, these findings create opportunities to diversify antigen selection for inclusion in cancer vaccines and cell-based therapies, with the ultimate goal of identifying and trafficking the most tumour-reactive T cells back to the T cell-excluded or T cell-deserted TME.

Adoptively transferred T cells

Adoptive T cell therapy has also been used to target TAAs directly, including in PDAC. Traditional targets include mesothelin and MUC1, although other TAAs such as CLA, CD318 and TSPAN8 have also been used as target antigens[143]. A phase I trial testing non-engineered T cells designed to simultaneously target PRAME, SSX2, MAGEA4, NY-ESO-1 and survivin in patients with metastatic PDAC demonstrated promising disease control, with 8 of 13 patients having stable disease for a longer-than-expected duration compared with historical control individuals[144]. The efficacy of adoptive T cells as monotherapy for patients with PDAC remains to be established. Nonetheless, this approach provides a method of priming an immunologically cold TME, with the potential for the T cells to be expanded ex vivo and/or engineered to become more reactive.

Targeting innate immunity

Most immunotherapies have focused on promoting the cytotoxic activity of effector T cells, although effectiveness is limited by non-inflamed tumours characterized by a paucity of infiltrating T cells, highlighting the need to identify additional mechanisms supporting conversion into hot tumours[145]. In particular, activation and maintenance of durable T cell responses is highly dependent on the innate immune system, which provides the first line of defence against microbial infections by monitoring for pattern recognition receptors to detect conserved structures on pathogens[146] (Fig. 4). Upon activation, the innate immune system, which primarily comprises macrophages, monocytes, DCs, polymorphonuclear cells and NK cells, mounts its own set of effector responses while also activating adaptive immunity, providing a unique point of convergence, as well as an opportunity for therapeutic intervention[147].

Data from several studies indicate that ligation of pattern recognition receptors, such as TLRs, cGMP–AMP synthase (cGAS) and retinoic acid-inducible gene-1-like receptors, can promote the activities of DCs and thus support antitumour immunity[147]. TLR3 is expressed on DCs and macrophages and specifically recognizes double-stranded RNA[148]. Activation of TLR3 signalling leads to increased production of type I IFNs and other pro-inflammatory cytokines via the transcription factors IFN regulatory factor 3 and nuclear factor-κB (NF-κB)[149]. Polyinosinic-polycytidylic acid (poly-IC) and its enhanced form, poly-ICLC, are TLR3 agonists capable of promoting DC migration to tumour-draining lymph nodes, leading to improved T cell priming, and this approach is well tolerated when administered concurrently with DC vaccines in patients with advanced-stage PDAC[150]. Rintatolimod, a TLR3-specific agonist, demonstrated an improved median OS (19.0 months versus 12.5 months; HR 0.51, 95% CI 0.28–0.90; $P = 0.016$) in patients with advanced-stage PDAC compared with matched individuals participating in a named patient programme[151,152]. Rintatolimod is currently being evaluated following FOLFIRINOX[153] or in combination with the anti-PD-L1 antibody durvalumab (NCT05927142) in patients with advanced-stage PDAC and preliminary results indicate that this approach is adequately tolerated. Other TLRs have also been evaluated as targets of novel immunotherapies. The physiological role of TLR7 and TLR8 is to detect single-stranded RNAs, whereas TLR9 senses unmethylated CpG dinucleotides[154]. BDB001, a novel TLR7/8 agonist, is being tested (alongside atezolizumab and stereotactic body radiotherapy (SBRT)) in patients with metastatic PDAC in the multicentre phase II AGADIR trial and reportedly met the primary end point of disease control (disease control rate 38%)[155]. Monotherapy with the TLR9 agonist IMO-2125 has been demonstrated to be effective in eliminating both local tumours and distant metastases by recruiting and activating DCs in a preclinical model of highly immunogenic PDAC. This agent was also found to be effective in combination with an anti-PD-1 antibody in models of less immunogenic PDAC[156]. SD-101 is a synthetic oligonucleotide with CpG motifs that activates TLR9 signalling in DCs, leading to increased type I IFN signalling, elevated antigen-specific $CD8^+$ T cell activity and synergy with anti-PD-1 antibodies in a preclinical model[157]. SD-101, alone or in combination with nivolumab and radiotherapy, has been tested in patients with locally advanced or metastatic PDAC[158,159]; however, results from this trial are currently not publicly available.

Upon detection of double-stranded DNA, cGAS produces cGMPs, which subsequently bind with stimulator of interferon genes (STING) and induce the synthesis of class I IFNs in DCs[160]. Synthetic cyclic dinucleotides were the first generation of STING agonists to enter clinical trials. Prior research has demonstrated that intratumourally injected STING agonists promote an inflamed TME and effector T cell infiltration, resulting in reductions in tumour burden in mouse models of PDAC[161,162]. Despite these promising preclinical results, the antitumour activity of STING agonists has not been translated into clinically effective therapies, partially owing to the challenge of intratumoural delivery of such agents. New-generation STING agonists suitable for systemic administration, including dazostinag (also known as TAK-676) and SNX281, have since been developed to overcome the high risk of systemic toxicities and thus avoid the issues associated with the need for intratumoural injection seen with first-generation designs[163–165]. The need to avoid intratumoural injections is particularly applicable to patients with PDAC owing to the difficulties associated with intratumoural administration both to primary and to metastatic lesions. BMS-986301, another second-generation STING agonist, has been tested in combination with the anti-PD-1 antibody nivolumab and the anti-CTLA4 antibody ipilimumab in patients with advanced-stage solid tumours (NCT03956680); preclinical data demonstrate similar antitumour activity and immunity with less T cell exhaustion when administered systemically, as opposed to intratumourally, in mouse models of PDAC[166].

Agents with the potential to target several aspects of the innate immune response simultaneously have also been explored[167]. Decoy20 is an attenuated, bacterial product capable of activating multiple endogenous innate immune signalling pathways. A preliminary biomarker analysis of plasma samples from patients with advanced-stage solid tumours demonstrated the ability of a single dose of Decoy20 to induce broad immune cell activation with threefold or more increase

in circulating levels of several inflammatory mediators CD40 ligand (CD40L), granulocyte colony-stimulating factor, IFNγ and IL-2 (ref. 167).

In addition to the synthetic innate agonists, immunogenic cell death due to the cytotoxic effects of chemotherapy and/or radiotherapy can also release damage-associated molecular patterns, leading to activation of innate immunity and thus functioning as an 'in situ vaccination' to prime the TME[168] (Fig. 4). Data from several phase I and II trials have demonstrated the safety of radiotherapy combined with various ICIs in patients with advanced-stage PDAC[169–171]. Among these, a phase II trial testing SBRT in combination with nivolumab and ipilimumab demonstrated disease control (a RECIST-defined response or stable disease) in 20% of patients and a median progression-free survival and OS of 2.5 months and 4.2 months, respectively, in patients with chemotherapy-refractory, metastatic PDAC[170]. We conducted a single-arm, phase II trial testing the combination of neoadjuvant GVAX, pembrolizumab and SBRT in patients with locally advanced PDAC to investigate the potential for synergy between GVAX and radiotherapy in priming the TME for a response to anti-PD-1 antibodies[172]. Translational investigations of resection specimens obtained in this trial demonstrated that this approach promotes effector T cell infiltration, as well as bringing effector T cells closer to cancer cells, and that both findings are associated with improved OS[173,174].

Activating T cells in the TME

Priming T cells with tumour antigens in combination with ICIs would be expected to elicit robust antitumour immunity, although such effects might be limited by the functional status of T cells owing to the presence of an immunosuppressive TME. Many components of the PDAC TME, including the ECM, CAFs, T_{reg} cells and immunosuppressive myeloid cells, are able to suppress T cell function. As a result, TME-resident T cells might be inactive, exhausted or both. Investigations of the underlying reasons for the insufficient efficacy of GVAX plus nivolumab have highlighted the importance of intratumoural T cell activation status, as indicated by CD137/4-1BB expression, and T cell exhaustion status, as marked by LAG3 expression[98]. A preclinical study testing the combination of GVAX in combination with an anti-PD-1 antibody and an agonistic anti-CD137 antibody in a syngeneic mouse orthotopic model of advanced-stage PDAC led to testing of this triplet combination as neoadjuvant therapy for patients with resectable PDAC[175]. Results from this trial have demonstrated the ability of the CD137 agonist urelumab to enhance cytotoxic effector T cell infiltration. This trial demonstrated a notable improvement in disease-free survival with the triplet combination of GVAX, nivolumab and urelumab compared with GVAX plus nivolumab only (median 33.5 months versus 15.0 months; HR 0.51, 95% CI 0.19–1.35; $P = 0.17$). Although the sample size was too small to draw definitive conclusions, 3 of 10 patients had pathological responses to a single cycle of treatment with the triplet combination, warranting further investigations[176]. Translational analysis of samples from this study further suggests that tumour-associated neutrophils (TANs) can contribute to T cell exhaustion, underscoring the importance of targeting other immunosuppressive cells within the TME to sustain effector T cell activation[98] (Fig. 4).

Targeting immunosuppressive cells to modulate the immune TME

Reprogramming neutrophils. Neutrophils comprise a substantial proportion of circulating immune cells and are abundantly present in the PDAC TME. Most studies have associated the presence of increased numbers of neutrophils with inferior outcomes and more aggressive PDAC phenotypes[177–179]. However, the role of neutrophils, limited by their short (7–10-h) half-life and the resulting technical difficulties in capturing their native state within the TME, remains controversial[180]. Different phenotypes of TANs have been identified with distinct roles dependent on specific microenvironmental cues. TGFβ, which is found at high levels in the PDAC stroma, promotes a tumour-promoting 'N2' phenotype, whereas inhibition of TGFβ signalling promotes a tumour-restraining 'N1' phenotype[181]. Various neutrophil subsets defined by transcriptional or protein markers have also been identified within this classification[182,183]. Such plasticity is anticipated to enable neutrophils to dynamically adopt both pro-tumorigenic and anti-tumorigenic functions within the PDAC TME (Fig. 5).

Several mechanisms are involved in the antitumour effects of neutrophils. Neutrophils can directly kill cancer cells via the release of reactive oxygen species (ROS) and reactive nitrogen species, are able to mediate antibody-dependent cellular cytotoxicity of cancer cells, secrete TNF and can recruit other pro-inflammatory immune cells[184]. By contrast, neutrophil-derived ROS and proteases can cause tissue damage via the promotion of wound-healing processes, chronic inflammation and the transformation of epithelial cells to cancer cells[180]. Mutated *KRAS* has an established role in driving the secretion of CXCL1, CXCL2 and CXCL5, all of which are ligands for CXCR2, the main receptor responsible for neutrophil recruitment[185]. TANs have also been shown to mediate angiogenesis by amplifying the activity of vascular endothelial growth factor through the release of matrix metalloproteinase-9 (ref. 186). Certain pro-inflammatory cytokines secreted by neutrophils, such as IL-17, can induce the development of PDAC stem cell features in PanINs, thus driving the aggressiveness of the malignancy and affecting the likelihood of disease progression[187]. Moreover, the formation of neutrophil extracellular traps (NETs) in NETosis, which normally occurs in response to bacterial and fungal infection, can be induced in the TME by CXCR1 and/or CXCR2 (CXCR1/2) signalling, HMGB1 and oxidative free radical species and can drive the suppression of T cell function and metastatic dissemination[188]. NETs can trap ct cells and also shield tumour cells from cytotoxic immune cells[189]. In mouse models, DNA from NETs has also been shown to directly promote the formation of distant metastases in the liver or lungs via interactions with the transmembrane protein CCDC25 on cancer cells[190]. Besides NETs, neutrophils can directly interact with ct cells in the bloodstream to drive cell-cycle progression to support metastatic potential[191].

Neutrophils can also contribute to the immunosuppressive PDAC TME. For example, TANs produce substantial amounts of arginase 1, which depletes arginine, a crucial amino acid for T cell activation and proliferation[192]. Similarly, neutrophils also inhibit NK cell activity by releasing hydrogen peroxide and reducing the secretion of NK cell-promoting cytokines such as IL-18 (refs. 193,194). PD-L1 expression on activated neutrophils, which is driven by tumour-derived GM-CSF in gastric cancer, might also induce T cell exhaustion[195].

Given these findings, interventions targeting tumour-promoting TANs, involving either depletion or reprogramming, have been examined in PDAC. One approach involves targeting IL-8 and the IL-8 receptors, CXCR1 and CXCR2 (refs. 196,197). TANs are likely to have contributed to T cell exhaustion in patients receiving GVAX plus nivolumab and urelumab in the previously described phase II trial[176]. Therefore, an anti-IL-8 antibody (NCT02451982) and SX-682 (NCT05604560), an orally bioavailable small-molecule inhibitor of CXCR1/2, are currently being tested in combination with anti-PD-1 antibodies in patients with resectable PDAC.

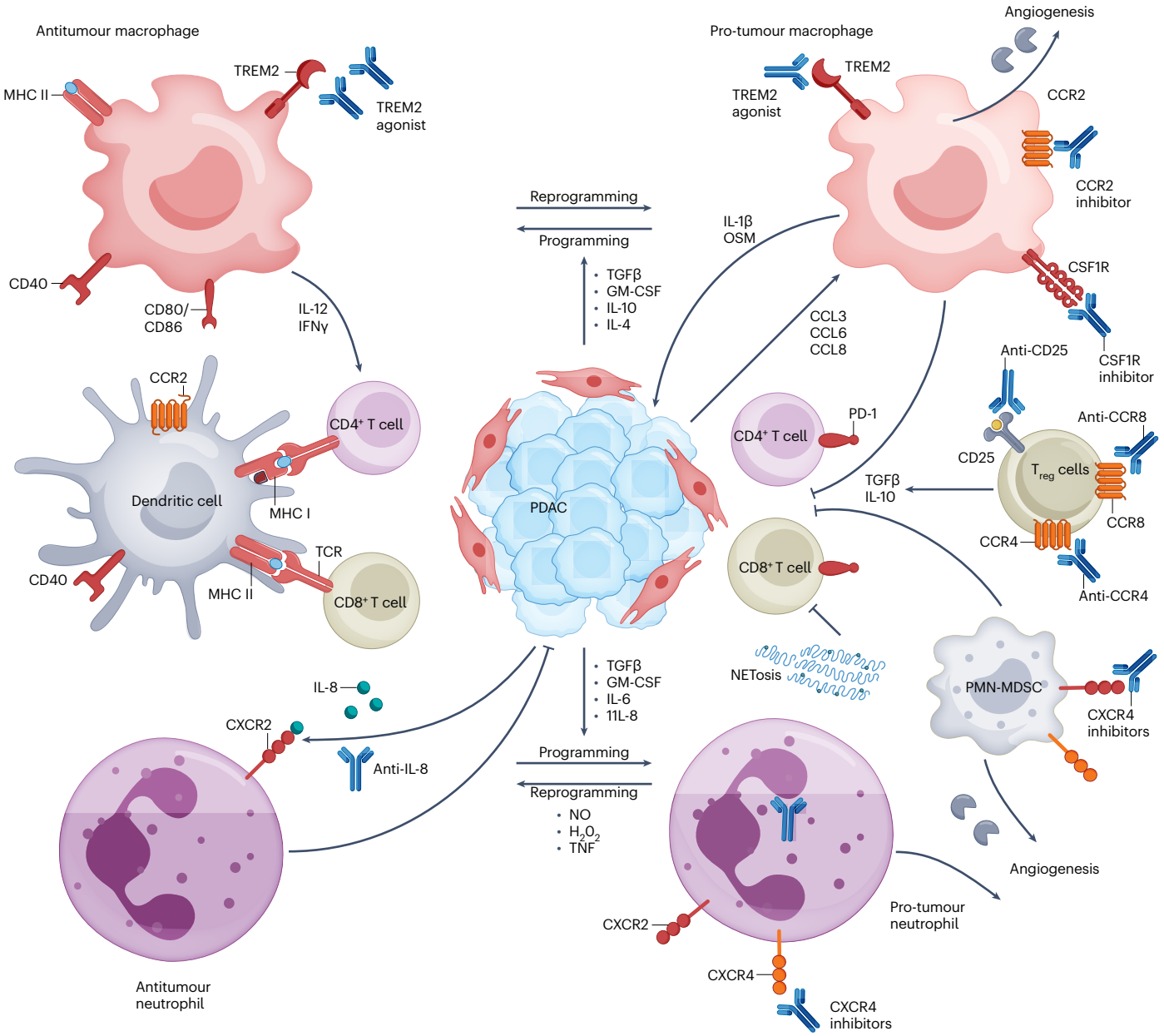

Fig. 5 | Reprogramming of immunosuppressive cells within the pancreatic ductal adenocarcinoma tumour microenvironment. Pancreatic ductal adenocarcinoma (PDAC) cells are capable of programming neutrophils and macrophages within the tumour microenvironment (TME) to a pro-tumour phenotype via the release of tumour-promoting inflammatory mediators such as granulocyte–macrophage colony-stimulating factor (GM-CSF), transforming growth factor-β (TGFβ), IL-6 and IL-10. These pro-tumour myeloid cells can promote angiogenesis and stromal remodelling, as well as driving effector T cell dysfunction by depleting arginine within the TME and secreting TGFβ and IL-10. Neutrophils can also secrete neutrophil extracellular traps (NETs) by NETosis, which can inhibit antitumour T cell responses. Macrophages can also directly produce IL-1β to drive inflammation, epithelial cell transformation and tumour progression. Beyond depleting these immunosuppressive cells with CCR2 inhibitors, these pro-tumour myeloid cells can potentially be reprogrammed to an immune-activating and antitumour phenotype through various mechanisms, including colony-stimulating factor 1 receptor (CSF1R) inhibitors, triggering receptor expressed on myeloid cells 2 (TREM2) agonists, CD40 agonists and anti-IL-8 monoclonal antibodies. Other immunosuppressive cells within the TME include myeloid-derived suppressor cells (MDSCs), which can be targeted using C-X-C motif chemokine receptor 2 (CXCR2) inhibitors. Regulatory T (T_{reg}) cells can also be targeted with different monoclonal antibodies targeting specific cell-surface proteins that promote the recruitment and/or immunosuppressive functions of T_{reg} cells. CCL, C-C motif chemokine ligand; CCR2, C-C motif chemokine receptor 2; CCR4, C-C motif chemokine receptor 4; CCR8, C-C motif chemokine receptor 8; CXCR4, C-X-C motif chemokine receptor 4; IFNγ, interferon-γ; OSM, oncostatin-M; PMN, polymorphonuclear; TCR, T cell receptor; TNF, tumour necrosis factor.

The CXCL12–CXCR4 signalling axis is another target of interest for neutrophil-related interventions[198] (Fig. 5). Accumulating evidence indicates a crucial role of CXCL12 in the homeostasis and recruitment of neutrophils to the TME[199,200]. The triplet combination of stromal hyaluronan degradation via PEGPH20, inhibition of focal adhesion kinase and an anti-PD-1 antibody has been shown to reduce the extent of polymorphonuclear MDSC (PMN–MDSC) and CXCR4$^+$ neutrophil infiltration in a syngeneic and orthotopic mouse model of PDAC[201]. Furthermore, the subsequent addition of an anti-CXCR4 antibody substantially reduced the incidence of liver metastases in these mice[201]. However, a phase II trial testing plerixafor, a small-molecule antagonist of CXCL12–CXCR4, in combination with the anti-PD-1 antibody cemiplimab, had only minimal efficacy, despite increased infiltration of myeloid cells into liver metastases. These findings suggest that either a more potent CXCR4-targeting agent or additional therapies, such as a focal adhesion kinase inhibitor, will be necessary for efficacy[202]. BL-8040, a synthetic peptide with a high affinity for CXCR4, has been tested in multiple trials involving patients with PDAC[203,204]. A phase IIa trial testing this agent showed that BL-8040 decreases MDSC and T_{reg} cell infiltration while increasing CD8$^+$ T cell infiltration within the TME[205]. In the expansion cohort of this trial, patients received BL-8040 plus the anti-PD-1 antibody pembrolizumab in combination with chemotherapy, resulting in an encouraging ORR of 32%, with disease control in 77% of patients[205].

Targeting TANs via inhibition of IL-8–CXCR2 or CXCL12–CXCR4 provides an appealing, albeit not clinically validated, method of functionally reprogramming tumour-promoting TANs towards an antitumour phenotype. In a study using an autochthonous mouse model of uterine cancer, the administration of respiratory hyperoxia as a means to improve tumour oxygenation resulted in a reduction in the extent of neutrophil recruitment, with the remaining infiltrating neutrophils demonstrating the capacity for T cell-independent cancer cell killing[206]. Hypoxia is a characteristic feature of the PDAC TME with a role in promoting neutrophil influx and the release of NETs; therefore, targeting the mechanisms underlying hypoxia-induced NETosis and neutrophil influx in the TME might lead to functional reprogramming of TAN phenotypes with subsequent enhancement of antitumour immunity[207–209] (Fig. 5).

Targeting MDSCs. Granulocytic MDSCs are a subset of immature myeloid cells that mediate the development of an immunosuppressive TME[210], as well as promoting cancer cell migration and formation of the premetastatic niche[211,212]. Similar to neutrophils, MDSCs can inhibit T cell activation indirectly via the production of arginase 1 and directly via the secretion of ROS[213,214]. Particularly, PMN-MDSCs, which are distinct from monocytic MDSCs (M-MDSCs) in terms of both cell surface marker expression and function (described in detail elsewhere[215]), account for the majority of tumour-infiltrating MDSCs, although distinctions between these cells and TANs in patients with cancer remains controversial. Genetic or pharmacological inhibition of CXCR2 (ref. 216), GM-CSF[217,218] or Ly6G[219] reduces the extent of MDSC infiltration and promotes adaptive antitumour immunity in mouse models, supporting the therapeutic potential of MDSC-targeted therapies. Data published in 2023 indicate that PDAC cells harbouring *KRAS* and *TP53* mutations have higher levels of CXCL1 expression, which recruits CXCR2$^+$ PMN-MDSCs to the TME and subsequently leads to the exclusion of CD8$^+$ T cells[220]. Interestingly, TNF secreted by recruited neutrophils further promotes CXCL1 production in cancer cells and CAFs, creating a feedforward loop that further drives CAF polarization towards an IL-6-secreting iCAF phenotype as well as sustained stromal inflammation and an immunosuppressive TME[220]. Disruption of such a loop via TNFR2 inhibition has been shown to increase sensitivity to chemotherapy, implicating TNFR2 as a promising therapeutic target for interventions targeting the downstream signalling pathways of MDSCs[220].

Reprogramming macrophages. Besides neutrophils, macrophages have long been known to have a pivotal role in both the tumorigenesis of PDAC and the promotion of an immunosuppressive TME[217,218,221,222]. Macrophages contribute to an inflammatory loop that drives epithelial cell transformation and cancer progression. Physical proximity of IL-1β$^+$ tumour-associated macrophages (TAMs) to neoplastic cells early in pancreatic tumorigenesis, elicited by tumour-derived prostaglandin E2, promotes an inflammatory transcriptional programme and causes neoplastic cells to acquire pathogenic properties[223]. Blockade of either prostaglandin E2 or IL-1β reprogrammes TAMs and subsequently suppresses inflammation in the pancreas, leading to tumour control in mouse models of PDAC and highlighting a possible preventative or therapeutic strategy targeting both immune dysregulation and tumour-promoting inflammation[223]. Macrophages also drive tumour cell growth and metastasis by secreting oncostatin M, which induces a more iCAF phenotype[224].

High levels of TAM infiltration have been associated with inferior outcomes in patients with PDAC, although these cells are a heterogeneous population capable of transitioning between multiple states between M1-like and M2-like polarization[225,226]. TAMs have historically been placed on a continuum of pro-inflammatory, antitumour M1-like macrophages to immunosuppressive, tumour-promoting M2-like macrophages[227]. M2-like TAMs are major reservoirs of cytokines and chemokines, capable of regulating the properties of other cellular stromal components and thus maintaining an immunosuppressive TME. Immunosuppressive TAMs secrete factors such as TGFβ and IL-10 as well as expressing exhaustion inducers such as PD-L1 that are able to dampen antitumour immune responses within the TME. However, this classification provides an oversimplified view of the complexity of macrophage polarization.

Multiple TAM phenotypes, including secreted phosphoprotein 1$^+$ and complement-1 Q$^+$ component TAMs, have been identified and associated with distinct outcomes in patients with PDAC[228,229]. Another major subpopulation of PDAC TAMs, characterized by the expression of triggering receptor expressed on myeloid cells 2 (TREM2), has garnered substantial attention across multiple malignancies[230]. Earlier studies demonstrated immunosuppressive effects of TREM2$^+$ TAMs, with depletion of TREM2$^+$ macrophages remodelling the myeloid immune landscape, curbing tumour growth and enhancing the efficacy of ICIs in mouse models of sarcoma, colorectal cancer or breast cancer[228,231]. Conversely, depletion of TREM2$^+$ macrophages was found to promote the development of hepatocellular carcinoma and glioblastoma in other studies[232,233]. Genetic depletion of *TREM2* in mouse models that spontaneously develop PDAC accelerates tumour progression by promoting pro-inflammatory macrophages and pathogenic inflammation, as mediated via NLRP3–NF-κB–IL-1β signalling[234]. These conflicting results suggest that the optimal approach might be to reprogramme TREM2$^+$ macrophages to enhance antitumour immunity while also maintaining their tumour-restraining functions.

The data described earlier raise concerns over interventions involving direct macrophage depletion, although targeting the CCL2–CCR2 and CSF1R signalling pathways, which regulate the recruitment of

TAMs to the TME, has been extensively explored[235]. Data from multiple preclinical studies demonstrate that CCR2 blockade can reduce TAM recruitment, enhance the efficacy of chemotherapy and reshape the immune microenvironment to amplify the antitumour activity of T cells[236–238]. The CCR2 agonist PF-04136309 demonstrated preliminary activity in a phase Ib trial involving patients with borderline resectable and locally advanced pancreatic cancer; however, a subsequent study in the same setting found no significant clinical benefit compared with chemotherapy alone as well as an increased incidence of pulmonary toxicities[239,240]. In addition to CCL2–CCR2, the CCL5–CCR5 axis is involved in the recruitment of both TAMs and T_{reg} cells. Our group thus examined the ability of the CCR2/CCR5 dual antagonist BMS-687681 to counteract radiotherapy-induced TAM recruitment into the PDAC TME in a mouse model and found that reduced TAM infiltration leads to increased expression of the effector T cell trafficking factors CCL17 and CCL22 (ref. 241). These findings led to a phase I/II trial combining radiotherapy with the CCR2/CCR5 antagonist BMS-813160 and nivolumab in patients with locally advanced PDAC, with preliminary evidence suggesting tolerability, with phase II data pending[242]. Various CSF1R inhibitors have also been shown to reduce macrophage infiltration, promote the recruitment of $CD8^+$ effector T cells, enhance chemosensitivity and synergize with ICIs in preclinical models[236,243]. However, deleterious effects on DCs, subsequently resulting in suppression of antigen presentation, remain a concern with approaches targeting bone marrow-recruited macrophages in general, with either a CCR2 inhibitor or a CSF1R inhibitor.

By contrast, other therapies have sought to reprogramme TAMs to become immune-stimulatory and thus tumour-restrictive, instead of directly inhibiting TAM recruitment (Fig. 5). CD40, a cell-surface marker and member of the TNF receptor superfamily, has been investigated as a target for monocyte reprogramming[244]. Upon interactions with CD40L, which is highly expressed by activated $CD4^+$ T cells, CD40 promotes upregulation of MHC II, co-stimulatory factors and IL-12 in antigen-presenting cells[245,246]. Activation of CD40 signalling with agonistic anti-CD40 monoclonal antibodies in macrophages induces the expression of pro-inflammatory genes, re-educates TAMs towards an tumour-restraining phenotype and drives macrophage-dependent antitumour effects in tumour-bearing mice[247,248]. Furthermore, CD40 agonists are capable of inducing DC-dependent antitumour immune responses when combined with chemotherapy, which triggers cancer cell apoptosis and TAA release[249]. A wide range of CD40 agonists, including SEA-CD40, selicrelumab, APX005M and CDX-1140, are under clinical investigation. Data from the phase Ib/II OPTIMIZE-1 trial demonstrate that mitazalimab, a human CD40 agonistic IgG1 antibody, administered in combination with mFOLFIRINOX, is tolerable and resulted in an objective response rate of 40% in patients with treatment-naive metastatic PDAC[250]. Also of note, a phase Ib trial combining sotigalimab with gemcitabine plus nab-paclitaxel, with and without nivolumab, initially demonstrated a promising objective response rate of 58% in patients with metastatic PDAC[251], although the subsequent phase II PRINCE trial testing this regimen in a larger cohort did not meet the 1-year OS primary end point[252]. These findings suggest that CD40 agonists might only be effective at promoting antigen presentation and need to be combined with a vaccine-type approach such as an oncolytic virus or a DC-based vaccine, which is currently being tested, respectively, in ongoing trials (NCT04787991 and NCT05650918)[3]. Beyond CD40, oncostatin M could serve as a target for macrophage reprogramming as mice deficient in this protein have improved macrophage-mediated antigen presentation as well as increased expression of T cell co-stimulatory receptors and activation markers such as CD137, CD44 and CD127 (ref. 224).

Several studies have differentiated among bone-marrow-derived TAMs, inflammatory monocytes and embryonically derived tissue-resident macrophages (TRMs). Monocyte-derived TAMs are involved in tumour antigen presentation, whereas embryonically derived TRMs have a profibrotic transcriptional profile[253]. Interestingly, a substantial fraction of macrophages accumulating in the PDAC TME is expanded from TRMs[253]. During the development of pancreatitis, TRMs in the pancreas trigger the accumulation and activation of fibroblasts, thus initiating the fibrosis required for wound-healing processes. However, loss of this protective mechanism owing to TRM depletion inhibits acinar cell survival, thus exacerbating pancreatitis[254]. This same TRM-elicited fibrotic mechanism is hijacked by the tumour to drive PDAC pathogenesis and progression[254]. TRMs can have tumour-promoting effects via other mechanisms, such as coordinating T_{reg} cell responses or promoting the growth of metastatic lesions in mouse models of lung adenocarcinoma and ovarian cancer, respectively[255,256]. Therefore, TRMs might be a better therapeutic target than recruited macrophages; however, they should be reprogrammed instead of depleted by therapeutic interventions.

Resistance arising from compensatory effects mediated by untargeted myeloid cell populations provides another major barrier to the effective therapeutic targeting of macrophages[219]. For example, depletion of $CXCR2^+$ TANs or $CCR2^+$ TAMs individually leads to a compensatory increase in the other myeloid subset, recapitulating the situation in patients with PDAC who received a CCR2 inhibitor plus mFOLFIRINOX in a phase Ib trial[257]. As such, CD11b has been considered as a target given its cell-surface expression across various immunosuppressive subsets of myeloid cells, including granulocytes, MDSCs and macrophages[258,259]. A novel small-molecule CD11b agonist GB1275 (formerly ADH-503) was demonstrated to partially activate CD11b, resulting in TAM repolarization and a reduction in immunosuppressive myeloid cell infiltration, improved DC activity and promotion of antitumour T cell cytotoxicity with sensitization to anti-PD-1 antibodies in a mouse model of PDAC[260,261]. Nonetheless, a phase I trial testing this CD11b modulator GB1275 either as monotherapy or combined with pembrolizumab in patients with solid tumours was terminated owing to a lack of efficacy, suggesting that targeting the entire myeloid cell population is unlikely to be clinically effective[262]. In summary, the ideal method of targeting TAMs within the TME should involve functional reprogramming from a tumour-promoting to an antitumour phenotype, with $TREM2^+$ macrophages and TRMs as the most appealing targets, based on preclinical data (Fig. 5).

Targeting T_{reg} cells

Within the lymphoid compartment, T_{reg} cells have a crucial role in driving and sustaining an immunosuppressive TME by directly lysing effector T cells, secreting immunosuppressive cytokines (such as TGFβ and IL-10) and expressing immune-checkpoints (such as TIGIT and CTLA4), resulting in dysfunctional DC maturation, antigen presentation and T cell activation[263]. T_{reg} cells accumulate during PDAC tumorigenesis[264], and higher levels of T_{reg} cells have been associated with an inferior prognosis[265]. An analysis of samples obtained from patients receiving GVAX showed that suppression of T_{reg} cell-associated signalling pathways and upregulation of T_H17 pathway components within PDAC lymphoid aggregates are associated with improved outcomes[97]. However, studies involving in vivo T_{reg} cell depletion have yielded contradictory results, albeit in different mouse models. For example, in one

study involving an orthotopic mouse model of *KRAS*^{G12D}-mutant PDAC, T_{reg} cell depletion slowed tumour progression, enhanced antitumour immunity, counteracted T_{reg} cell-restrained DC expansion and increased the expression of co-stimulatory molecules[266]. By contrast, research by another group showed that T_{reg} cell depletion in a genetically engineered autochthonous mouse model leads to accelerated tumour progression, a loss of TGFβ-driven, tumour-restraining CAFs and an increase in myeloid cell-mediated compensatory immunosuppression via CCL3, CCL6 or CCL8 signalling[267]. These studies collectively reveal the complex roles of T_{reg} cells in the TME and highlight the challenges associated with interventions targeting these cells.

Therapies targeting T_{reg} cells, including low-dose cyclophosphamide[268] and monoclonal antibodies targeting cell-surface proteins, have traditionally involved either T_{reg} cell depletion or prevention of T_{reg} cell infiltration into tumours. Particularly, T_{reg} cells express high levels of CD25, although previous attempts to deliver systemic CD25-targeted therapy have also resulted in depletion of CD25$^+$ effector T cells[269,270]. To overcome this challenge, investigators developed vopikitug, a novel non-IL-2-blocking anti-CD25 antibody designed to specifically deplete T_{reg} cells while preserving IL-2–STAT5 signalling in effector T cells[271]. Nonetheless, and despite promising preclinical activity and the induction of measurable intratumoural T_{reg} cell depletion in a phase I trial involving patients with advanced-stage solid tumours, clinical activity was limited to partial responses in 3 of 49 patients who received vopikitug plus atezolimumab, thus precluding further clinical testing[272]. Recruitment of T_{reg} cells into the TME is regulated by interactions such as the CCL22–CCR4 and CCL1–CCR8 signalling[269], and several monoclonal antibodies targeting CCR4 (ref. 273) and CCR8 (refs. 274,275) on T_{reg} cells have been tested in preclinical studies, with evidence suggesting preclinical activity. The CCR4 antagonist tivumecirnon (NCT04768686, NCT04894994 and NCT03674567), the anti-CCR8 monoclonal antibody denikitug (NCT05007782) and the anti-CCR8 antibody S-531011 (NCT05101070) are currently being investigated either as single agents or in combination with ICIs in patients with various advanced-stage solid tumours. Once the activity of these agents is confirmed in these non-pancreatic solid tumours, exploration of their potential clinical utility in modulating the PDAC TME is warranted.

ICIs targeting CTLA4 can inhibit the interactions between CTLA4 and CD80/CD86, leading to enhanced CD28 co-stimulation in effector T cells[276]. Interestingly, although anti-CTLA4 antibodies have been shown to deplete T_{reg} cells via Fc-mediated antibody-dependent cellular cytotoxicity in mouse models, this effect is not observed in patients receiving either ipilimumab or tremelimumab[277]. This observation might be explained by an inhibitory Fc receptor FcγRIIB, which can be found in both humanized mouse models and patients, that might have inhibited the effects of these anti-CTLA4 antibodies, a hypothesis further supported by the development of Fc-engineered antibodies with minimized FcγRIIB binding activity and a substantially enhanced capacity for T_{reg} cell depletion[278]. Other anti-CTLA4 antibodies[279], including botensilimab[280], have leveraged improved Fc engineering for enhanced therapeutic efficacy, highlighting the potential of this approach in depleting T_{reg} cells and inhibiting T_{reg} cell activity in the TME.

Ultimately, although T_{reg} cells probably have a crucial role in driving the immunosuppressive TME in PDAC, direct targeting of these cells has proven difficult, suggesting that a more effective approach would be to target other mechanisms within the TME that could suppress T_{reg} cell infiltration, reshape the T cell landscape and/or alter T_{reg} cell function[281]. Indeed, in the future, even highly effective T_{reg} cell-targeting agents will probably need to be paired with additional therapies that can prime and activate T cells within the TME for optimal efficacy.

Remodelling the TME via metabolic reprogramming

The desmoplastic stroma of PDAC limits access to blood-derived oxygen and nutrients, creating a unique metabolic milieu featuring an abundance of certain metabolites and a scarcity or absence of others[282,283]. This altered nutrient access forces both tumour and stromal cells to adapt to the nutritional constraints imposed by the PDAC TME and offers a point of convergence that enables the targeting of multiple components with a potential role in tumorigenesis and disease progression. Numerous studies have investigated the metabolic alterations intrinsic to PDAC cells and identified potential targets, as described in detail elsewhere[284–286]; therefore, we particularly focus on metabolic crosstalk involving both cancer cells and other TME components.

Glutamine has a crucial role in the desmoplastic reaction and modulation of the immune microenvironment, providing a point of biological convergence for several therapeutic strategies[287]. Specifically, inhibition of glutamine metabolism substantially reduces the activity of the hexosamine biosynthesis pathway with subsequent reductions in collagen and hyaluronan deposition and increased CD8$^+$ T cell infiltration[288,289]. Elsewhere, disruption of glutamine–glutamate cycling either by inhibiting glutamine synthetase directly or by inhibiting the synaptic protein vesicular glutamate transporter 1 has been shown to reduce the secretion of tumour-promoting cytokines by CAFs[61]. Furthermore, glutamine antagonism with 6-diazo-5-oxo-l-norleucine (DON), which inhibits several glutamine-requiring enzymes, has been shown to promote the activity of CD8$^+$ effector T cells by restoring glutamine availability in the TME and potentiates the antitumour activity of anti-PD-1 antibodies in mouse models[289,290].

The effects of blockade of glutamine metabolism on myeloid cells are less well understood. Inhibition of glutamine metabolism has been demonstrated to decrease the generation and recruitment of immunosuppressive MDSCs by reducing tumour-derived CSF-3 secretion while also increasing the expression of MHC II and CD80 on TAMs[291,292]. Interestingly, cancer cells and DCs compete for glutamine uptake via the cell membrane transporter SLC38A2; thus, the effects of inhibiting glutamine metabolism on DC function are likely to be difficult to predict. Indeed, intratumoural glutamine supplementation has been shown to augment the activation of type 1 conventional DCs (cDC1s) and to promote cDC1-mediated CD8$^+$ T cell antitumour immunity[293]. Conversely, data from another study indicate that inhibition of glutamine metabolism with DON decreases the proliferation and survival of cDC1s[294]. These preclinical data highlight the complexity of glutamine metabolism in immune responses and suggest the need for specific inhibition of glutamine metabolism in cancer cells and immunosuppressive cells, but not necessarily in other cells of the TME[294]. Besides glutamine, other major metabolites including glucose, alanine[295–297], collagen[298] and ribose[299] have crucial roles in tumour–stroma crosstalk[300] and are currently being investigated further. For example, we found that PDAC cells are able to epigenetically reprogramme glucose metabolism in M1-like macrophages to favour a tumour-promoting phenotype via glycoprotein A repetitions predominant, a TGFβ-activating protein that promotes immune escape and dissemination[301] (Fig. 6).

Autophagy is another major targetable point of metabolic convergence (Fig. 6). This self-eating cellular recycling programme is crucial for tumorigenesis and disease progression in mouse models of PDAC[302–304]. Genetic or pharmacological inhibition of autophagy

leads to robust tumour regression and improved survival in several of these preclinical models[305–307]. Furthermore, data from multiple trials demonstrate the safety of autophagy inhibition with hydroxychloroquine, although efficacy remains uncertain and phase II trials involving patients with PDAC have thus far failed to demonstrate a significant improvement in OS when hydroxychloroquine is added to standard-of-care chemotherapy[286,308–310]. However, autophagy also has a crucial role in immune evasion, potentially providing another target for remodelling of the tumour immune microenvironment[311]. MHC I molecules in PDAC cancer cells undergo lysosomal degradation in an autophagy-dependent manner via the autophagy cargo receptor NBR1 (ref. 312). Thus, instead of being presented at the cell surface, MHC I molecules are predominantly localized to autophagosomes and lysosomes. Inhibition of autophagy (either genetically or pharmacologically using chloroquine) promotes cell-surface MHC I expression and antigen presentation, as well as CD8$^+$ T cell-mediated immunity with evidence of synergy with anti-PD-1 and anti-CTLA4 antibodies[312]. In a separate study using orthotopic syngeneic mouse models of PDAC, the combination of chloroquine plus an FLT3 ligand induced CD8$^+$ T cell exhaustion with increased expression of LAG3, whereas the addition of an anti-LAG3 antibody to this combination substantially reduced tumour growth[313]. These studies provide a preclinical rationale for combining autophagy inhibition with ICIs as a therapeutic strategy against PDAC.

Other metabolic mechanisms of interest within the PDAC TME include dysfunctional lipid and glucose metabolism. Despite the

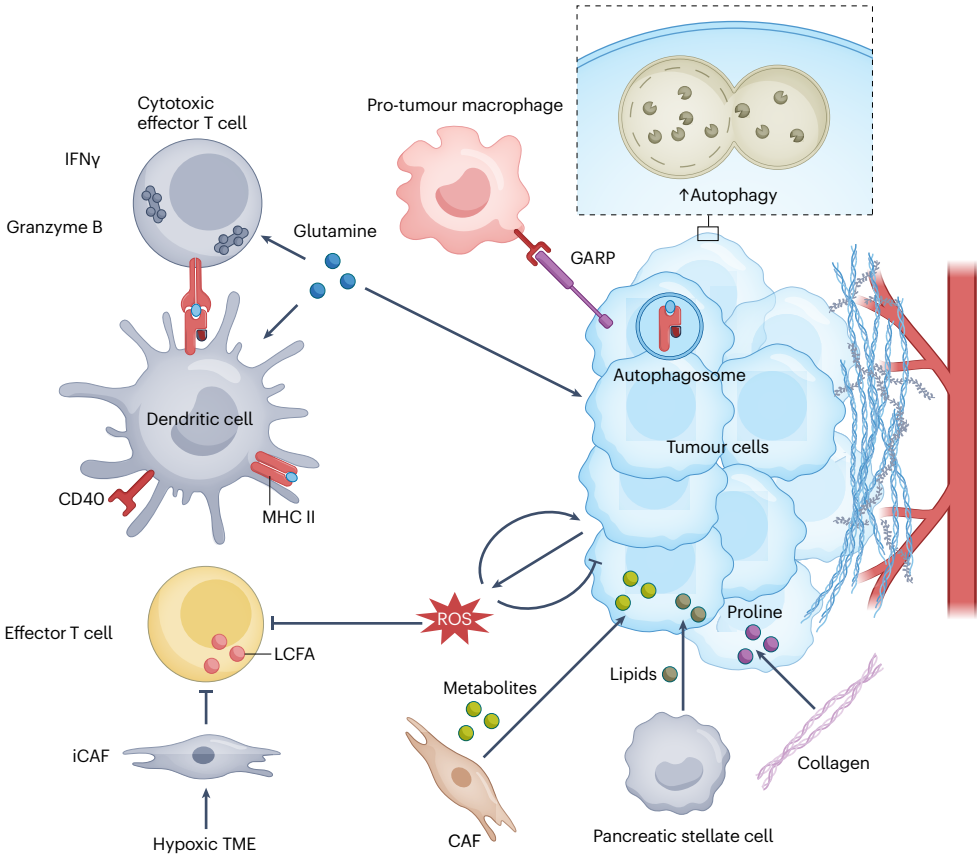

Fig. 6 | Metabolic convergence within the pancreatic ductal adenocarcinoma tumour microenvironment. The desmoplastic stroma of pancreatic ductal adenocarcinoma (PDAC) creates a unique metabolic milieu that is hypoxic and abundant in certain metabolites but lacking in others, forcing both cancer cells and stromal cells to adapt to the tumour microenvironment (TME). Glutamine is a particularly in-demand metabolite and is required for cancer cells to fulfil their biosynthetic needs, while also being consumed in the recruitment of myeloid-derived suppressor cells and inducing a pro-tumour cancer-associated fibroblast (CAF) phenotype. Glutamine is also required by CD8$^+$ T cells and dendritic cells to fulfil their antitumour functions, meaning that inhibition of glutamine metabolism specifically in cancer cells might simultaneously increase the supply of glutamine available to antitumour immune cells. Although CD8$^+$ T cells within the TME can still utilize long-chain fatty acids (LCFAs) as a nutrient source in the absence of glucose, accumulation of LCFAs in CD8$^+$ T cells can also drive metabolic dysfunction, thus inhibiting antitumour activity. M1-like macrophages can be reprogrammed to a pro-tumour phenotype via glycoprotein A repetitions predominant (GARP)-dependent modulation of glucose metabolism. Beyond having a crucial role in PDAC cell survival and proliferation, autophagy also downregulates the cell-surface expression of MHC I on the cancer cell surface, facilitating immune evasion and highlighting autophagy as a major targetable point of metabolic convergence. Neoplastic cells can also obtain nutrients such as lipids and other metabolites by rewiring pancreatic stellate cells and CAFs. Collagen can also act as a source of proline for PDAC cells within the nutrient-limited TME. The hypoxic TME can also induce an inflammatory CAF (iCAF) phenotype that inhibits T cell activity. Finally, more research is needed to understand the role of reactive oxygen species (ROS) within the TME, given the ability of these molecules to drive immune dysfunction and cancer cell proliferation while also being capable of inducing immunogenic cell death of cancer cells following accumulation to sufficiently high levels. IFNγ, interferon-γ.

generally nutrient-depleted TME, PDAC cells often have elevated levels of de novo lipogenesis[314,315] and fatty acid uptake[316], supporting continued growth and metastatic dissemination. Beyond these cell-intrinsic mechanisms, which have been described in detail elsewhere[317–319], lipids also have a crucial role in cellular crosstalk between different TME components and drive functional changes in the TME. For example, uptake of stromal-derived lysophosphatidylcholines by PDAC cells drives cancer cell migration and proliferation via a lysolipid–autotaxin signalling axis, which can be reversed by inhibition of autotaxin[320] (Fig. 6). The functional fate and 'fitness' of effector T cells are also highly dependent on nutrient availability within the TME[321]. Glucose is also essential for optimal T cell activation[322–324] but is scarce within the TME owing to the Warburg effect. Nevertheless, $CD8^+$ T cells should be able to maintain their proliferation and effector functions by adapting to generate energy via β-oxidation of long-chain fatty acids (LCFAs) to provide fuel for mitochondrial oxidative phosphorylation[325,326]. However, this mechanism is not supported by data from a separate study showing that lipids accumulate in the TME of a mouse model of PDAC and in intrapancreatic $CD8^+$ T cells[327]. Instead of utilizing LCFAs as an energy source, $CD8^+$ T cells in the PDAC TME were driven towards a metabolically exhausted state owing to downregulation of very-long-chain acyl-CoA dehydrogenase (VLCAD), which subsequently led to the accumulation of toxic levels of very-LCFAs and LCFAs[327] (Fig. 6). Metabolic reprogramming of these T cells via enforced expression of *ACADVL* (encoding VLCAD) led to improved intratumoural T cell survival and persistence and subsequently overcame resistance to adoptive T cell transplantation in mouse models of PDAC[327].

The PDAC TME is metabolically modulated by the generation of ROS under hypoxic conditions[328,329]. Hypoxia can induce an iCAF state through activation of IL-1 signalling and independent of HIF1α[330]. Nonetheless, the generation of ROS is a double-edged sword — ROS are able to promote cancer cell proliferation, EMT and invasion, although higher ROS levels can also induce immunogenic cell death and thereby activate antitumour immunity[331]. Data from studies in mouse models of various other cancers suggest that exposure to antioxidants or enhancing the expression of proteins with antioxidant effects can accelerate tumour progression and metastasis[332–336]. ROS act as crucial messengers during TCR activation and have important roles in functional immune regulation, with prolonged exposure to elevated ROS levels driving T cell dysfunction and inducing the accumulation of T_{reg} cells[337,338]. Thus, future studies will be needed for a better understanding of how ROS can be optimized at a level sufficient to induce cancer cell death while maintaining functional antitumour immunity. Together, combined with our understanding of the metabolic heterogeneity of PDAC in patients[339,340], preclinical findings in this area are anticipated to pave the way for the future development of novel therapies targeting the metabolic crosstalk that modulates the PDAC TME.

Targeting intrinsic and extrinsic mechanisms

Remodelling the TME by targeting mutant KRAS

KRAS is the most commonly mutated oncogene in PDAC, with >90% of these tumours harbouring a *KRAS* mutation[341]. Landmark studies in the past have demonstrated that oncogenic KRAS is capable of inducing the formation of PanINs and eventually invasive PDAC[342,343]. KRAS-G12D has been shown to regulate anabolic metabolism of glucose in a mouse model of PDAC equipped with inducible $KRAS^{G12D}$ by promoting glucose uptake and ribose biogenesis and shunting glucose intermediates into hexosamine and pentose phosphate pathways[344]. KRAS signalling is also known to alter the TME by promoting the development of the fibroinflammatory microenvironment necessary for PDAC cell survival[345]. Oncogenic KRAS signalling can also induce the expression of GM-CSF in PDAC cells to recruit immunosuppressive MDSCs[217,218]. In addition, mutant KRAS can drive the secretion of IL-10 and TGFβ via activation of the MEK–ERK–AP1 signalling pathway, leading to T_{reg} cell induction and M2 polarization of TAMs[346]. Mutant KRAS proteins can also regulate immune evasion by downregulating antigen presentation[347], driving T cell exhaustion through cytokine secretion, and by upregulating PD-L1 expression by increasing the stability of PD-L1 mRNAs[348] (Fig. 7).

Despite considerable drug development efforts, RAS was considered undruggable for decades owing to a lack of targetable deep hydrophobic pockets[349,350]. However, researchers have since developed small molecules capable of binding to cysteine 12 in the newly identified switch II region of the RAS protein[351]. These small-molecule inhibitors covalently bind to the GDP-bound 'OFF' form of KRAS-G12C[351,352]. Two KRAS-G12C inhibitors, adagrasib and sotorasib, have demonstrated clinical activity in patients with $KRAS^{G12C}$-mutant solid tumours[353,354] and several next-generation KRAS-G12C inhibitors, such as elirasib or the 'ON'-state inhibitors elironrasib and BBO-8520, are under clinical investigation[355–359]. However, given that $KRAS^{G12C}$ accounts for only 1–2% of all *KRAS* mutations observed in PDAC, an unmet need exists for inhibitors capable of targeting alterations more commonly observed in PDAC, such as KRAS-G12D and KRAS-G12V[360].

MRTX1133, an OFF-state KRAS-G12D inhibitor discovered using structure-based drug design, is the first non-covalent KRAS-G12D inhibitor to enter clinical testing[361]. This agent inhibits ERK1/2 phosphorylation and downstream mitogenic signalling, resulting in a reduction in PDAC cell proliferation[362,363]. However, the first-in-human trial testing MRTX1133 has been terminated early owing to pharmacokinetic issues, and a new formulation is being developed[364]. HRS-4642, another potent and selective KRAS-G12D inhibitor, has been demonstrated to be tolerable with preliminary evidence of antitumour activity in a phase I trial mostly involving patients with non-small-cell lung cancer and one patient with PDAC (including one partial response and stable disease in 11 of 18 patients)[365]. Data from a genome-wide CRISPR–Cas9 screen demonstrate that targeting the proteasome further sensitizes tumours to HRS-4642, and this finding was validated in mouse models exposed to the combination of HRS-4642 plus the proteasome inhibitor carfilzomib. HRS-4642, with or without carfilzomib, was also found to remodel the immune TME in mouse models of PDAC, with an increase in the proportion of effector ($CD44^+CD62L^-$) $CD4^+$ and $CD8^+$ T cells, suppression of macrophage infiltration and polarization of TAMs towards an M1 phenotype[365,366]. Other KRAS-G12D inhibitors, including the small-molecule inhibitor BPI-501836 (ref. 367) and the proteolysis-targeting chimeric degrader ASP3082 (ref. 368), are currently under clinical evaluation with preliminary evidence of activity of the latter in this setting.

Given the heterogeneity of *KRAS* mutations across not only PDAC but also other malignancies, development of pan-RAS inhibitors is an appealing objective. RMC-7977 is a pan-RAS inhibitor that targets both wild-type and mutant forms of RAS in the GTP-bound ON state, resulting in sustained suppression of ERK phosphorylation in PDAC cells[369]. Despite activity against both wild-type and mutant RAS, evidence from preclinical models suggests that RMC-7977 has limited adverse effects in non-malignant tissues[370]. More importantly, the nonspecific inhibition of KRAS by this agent enables RMC-7977 to overcome the upregulation of both wild-type and mutant KRAS in response to KRAS-G12C inhibitors[369]. The activity of RMC-7977 is also unaffected by secondary *KRAS* mutations

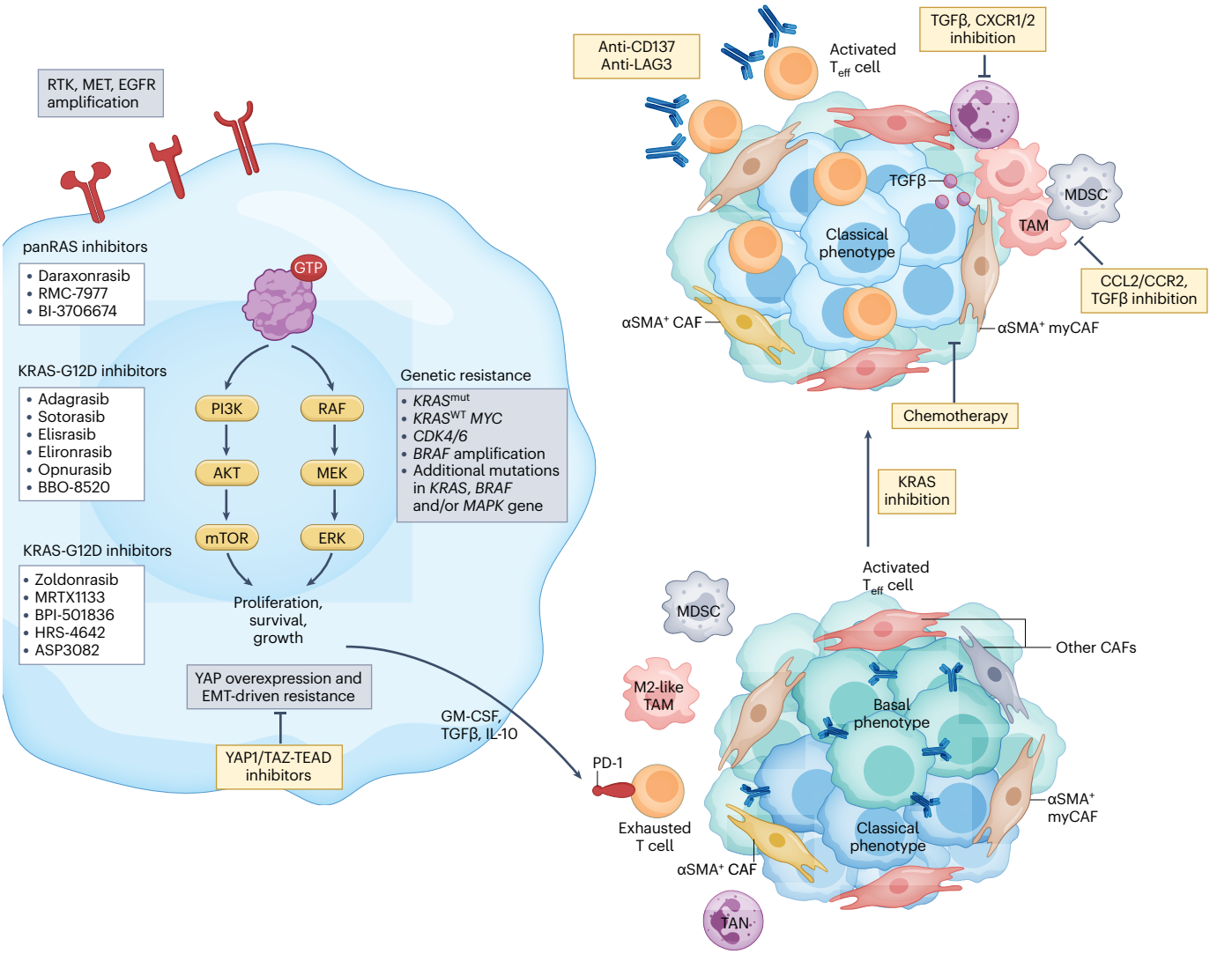

Fig. 7 | KRAS inhibition within the pancreatic ductal adenocarcinoma tumour microenvironment. Oncogenic KRAS signalling substantially alters the tumour microenvironment (TME) to provide a suitable niche for pancreatic ductal adenocarcinoma (PDAC) cells. This signalling axis, which includes input from granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-10, promotes the recruitment of immunosuppressive immune cells, drives exhaustion of antitumour immune effector cells and polarizes other immune and stromal cells towards a pro-tumour phenotype. Treatment options (in light purple boxes) that target oncogenic KRAS and the mechanisms of resistance (in dark purple boxes) are summarized. Beyond directly inhibiting cancer cell proliferation, KRAS inhibition also remodels the TME, including by increasing the infiltration of effector T cells (T_{eff}) and reprogramming cancer-associated fibroblasts (CAFs) to a potentially tumour-restrictive α-smooth muscle actin-positive myofibroblastic (αSMA$^+$ myCAF) phenotype. However, resistance to KRAS inhibition, for example, via amplifications or mutations in *KRAS*, *MYC*, *CDK4/6*, *BRAF* and/or other genes encoding components of the mitogen-activated protein kinase pathway, can develop following treatment. Furthermore, surviving cancer cells of a classical phenotype, which are more resistant to KRAS inhibition, can act as reservoirs for the subsequent development of resistant cancer cells enabling later disease recurrence. The accumulation of tumour-associated macrophages (TAMs), tumour-associated neutrophils (TANs) and/or myeloid-derived suppressor cells (MDSCs) can also drive immune exhaustion and resistance to KRAS inhibition. Thus, combining novel RAS inhibitors with chemotherapy and other immune-modulating therapies can effectively remodel the TME towards an antitumor phenotype and overcome treatment resistance. TME-targeting strategies that might overcome resistance to inhibitors of mutant KRAS are indicated (in yellow boxes). CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; CXCR1/2, C-X-C motif chemokine receptor 1 and/or 2; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; LAG3, lymphocyte activation gene 3; TAZ, transcriptional coactivator with PDZ-binding motif; TEAD, transcriptional enhanced associate domain; T_{reg}, regulatory T cell; TGFβ, transforming growth factor-β; YAP, Yes-associated protein.

affecting the binding ability of KRAS-G12C inhibitors, for example, at residues R68, Y96 and H95 in the switch II pocket[369]. A closely related pan-RAS inhibitor, daraxonrasib, showed promising results in a phase I/Ib trial including two objective responses, and this agent is now being evaluated versus second-line chemotherapy in a phase III trial involving patients with previously treated metastatic PDAC (NCT06625320)[371].

As anticipated, KRAS inhibitors are able to substantially alter the PDAC TME, in addition to their cancer cell-intrinsic effects. For example, MRTX1133 has been shown to upregulate antigen presentation and IFNγ signalling with downregulation of *TGFB1* expression on tumour cells as well as substantial reductions in the infiltration of immunosuppressive neutrophils accompanied by increased recruitment of $CD8^+$ T cells in a mouse model of PDAC[372]. KRAS-G12D inhibition can also skew TAMs towards an M1-like phenotype and increase the number of αSMA^+ myCAFs[363]. Studies testing MRTX1133 using a pancreas-specific $KRAS^{G12D}$-inducible ($iKRAS^{G12D}$) mouse model have uncovered an additional Fas-dependent antitumour mechanism beyond inhibition of intrinsic mitogenic signalling[373–375]. Mechanistically, KRAS-G12D epigenetically silences the expression of *Fas* in cancer cells by inducing hypomethylation of the promoter region, and KRAS-G12D inhibition restores *Fas* expression and thus promotes apoptosis of cancer cells mediated by binding of Fas ligand on $CD8^+$ T cells to Fas on cancer cells[373]. The apparent need for T cell-mediated immunity for KRAS inhibitors to achieve sustained tumour regression and disease control could limit the clinical effectiveness of these agents, given the low levels of T cell infiltration at baseline in many patients[363,374]. This observation also underscores the need to combine KRAS inhibitors with other stromal or immune-targeting agents such as KRAS-targeting vaccines to promote T cell recruitment.

Resistance to KRAS inhibitors

Despite the potential of KRAS inhibitors to inhibit cancer cell proliferation and alter the TME, resistance to these agents is a common occurrence and is poorly understood[376]. Most patients with advanced-stage $KRAS^{G12C}$-mutant solid tumours do not have a response to KRAS-G12C inhibitor monotherapy, and a deeper understanding of these mechanisms is necessary for patient stratification[377]. Furthermore, as with most targeted therapies, acquired resistance to these agents is almost universal and leads to disease relapse following a response. Analysing samples from a cohort of patients with $KRAS^{G12C}$-mutant PDAC who received adagrasib or sotorasib, investigators identified several common mechanisms of resistance, such as amplifications of $KRAS^{G12C}$, *MYC*, *MET*, *EGFR* and/or *CDK6*, in around half of all patients[378]. However, 54% of patients had no detectable genetic mechanisms of resistance, a finding that was recapitulated in a group of $KRAS^{LSL\text{-}G12D/+}$; $TP53^{LSL\text{-}R172H/+}$; p48-*Cre* (KPC) mice exposed to MRTX1133, suggesting that non-genetic mechanisms also have a role in resistance to KRAS-targeted therapies[378].

Different transcriptional cellular states are associated with distinct patterns of responses to KRAS inhibitors, potentially including some non-genetic mechanisms of resistance[379]. Interestingly, data from both in vitro and in vivo studies indicate that mesenchymal and basal-like cellular states are associated with improved responses to KRAS inhibitors, and data from lineage-tracing studies demonstrate that residual classical-like (as opposed to basal) PDAC cells enriched after KRAS inhibition become a reservoir for disease recurrence[380]. Thus, as anticipated based on this hypothesis, chemotherapy and MRTX1133 provide markedly improved tumour control in mouse models of PDAC[380]. Other studies using the $iKRAS^{G12D}$ model have shown that EMT[381], *YAP1* amplifications[382,383], adoption of an oxidative phosphorylation-dependent cell state[384], mesenchymal reprogramming via the SMARCB–MYC signalling pathway[385] and USP1-dependent upregulation of macropinocytosis[386] can also overcome oncogenic RAS addiction. Future studies will be required to determine whether these findings reflect resistance to pharmacological inhibition of KRAS in patients with PDAC.

Targeting the TME to overcome resistance to KRAS inhibitors

Beyond these cell-intrinsic mechanisms that enable cancer cells to escape dependency on mutant KRAS, and thus confer resistance to KRAS inhibitors, alterations in the TME might also enable such dependencies to be bypassed. For example, *HDAC5* overexpression was identified as a top hit among genes enabling mutant *KRAS*-independent tumour growth in the inducible $KRAS^{G12D}$;$TP53^{-/-}$ mouse model of PDAC[387]. Mechanistically, HDAC5 represses *SOCS3*, which leads to subsequent upregulation of CCL2 expression and macrophage recruitment, thereby resulting in a prominent switch from a neutrophil-dominated to a macrophage-dominated TME. These tumour-infiltrating macrophages secrete TGFβ, which enables cancer cells to bypass mutant KRAS dependency through an SMAD4-mediated mechanism, providing a rationale for combining CCL2/CCR2 or TGFβ inhibitors with KRAS inhibitors[387]. The combination of MRTX1133 plus a CXCR1/2 inhibitor, an anti-LAG3 antibody and an agonistic anti-CD137 antibody has been shown to induce marked tumour regression and prolong survival in an autochthonous mouse model of PDAC[388]. Thus, the potential of TME remodelling as a mechanism of bypassing dependency on mutant KRAS supports the further exploration of strategies targeting the TME either in combination with or following KRAS inhibitors to overcome or delay the onset of resistance to these agents (Fig. 7).

Conclusions

Over the past 5 years, considerable progress has been made in our understanding of the PDAC TME, although this progress has not been translated into substantial improvements in patient care. The identification of novel cellular subpopulations in the TME has enabled us to target tumour-promoting components more specifically by reprogramming these specific components, in contrast to untargeted depletion of entire TME components. Going forward, novel therapeutic strategies should focus on combination strategies targeting multiple specific TME components while also accounting for possible compensatory effects and mechanisms of resistance.

Our understanding of both intratumoural and intertumoural heterogeneity has highlighted the need to make informed therapeutic decisions based on the characterization of individual TMEs and the importance of more robust biomarker selection for patient stratification. We are now better placed than ever to potentially address these challenges following advances in single-cell spatial multi-omics and their analysis using machine learning-based models[389] as well as innovative approaches to protein characterization and drug design. With these technologies, we are equipped to make further progress in our understanding of the TME and build on our experiences with various failed clinical trials and develop more effective therapies for patients with PDAC.

Published online: 03 October 2025

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Acknowledgements

The work of L.Z. is supported by National Institutes of Health grants R01CA169702, R01CA197296 and P01CA247886; National Cancer Institute Specialized Programs of Research Excellence in Gastrointestinal Cancers grant P50 CA062924; Sidney Kimmel Comprehensive Cancer Center grant P30 CA006973 and Mays Cancer Center grant P30 CA054174.

Author contributions

H.-C.K., K.W.Z. and L.Z. researched data for the manuscript. H.-C.K. and L.Z. made substantial contributions to discussions of content. H.-C.K., J.W.Z. and L.Z. wrote the manuscript and reviewed and/or edited before submission.

Competing interests

L.Z. has acted as a consultant and/or adviser of Akrevia/Xilio, Alphamab, Amberstone, Ambrx Biosion, Clinicaltrial Option, Duo Oncology, Fortress Biotech, Histosonics, Mingruizhiyao, NovaRock, QED and Tavotek, has received research funding from Abmeta, AstraZeneca, Bristol-Meyer Squibb and Merck and holds shares in Alphamab, Amberstone, Cellaration and Mingruizhiyao. J.W.Z. has received research funding from Roche Genentech. The other authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Clinical Oncology* thanks P. Philips, who co-reviewed with G. Sharbeen; and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.