Cancer-associated fibroblasts in pancreatic cancer: new subtypes, new markers, new targets

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Abstract

Cancer-associated fibroblasts (CAFs) have conflicting roles in the suppression and promotion of cancer. Current research focuses on targeting the undesirable properties of CAFs, while attempting to maintain tumour-suppressive roles. CAFs have been widely associated with primary or secondary therapeutic resistance, and strategies to modify CAF function have therefore largely focussed on their combination with existing therapies. Despite significant progress in preclinical studies, clinical translation of CAF targeted therapies has achieved limited success. Here we will review our emerging understanding of heterogeneous CAF populations in tumour biology and use examples from pancreatic ductal adenocarcinoma to explore why successful clinical targeting of protumourigenic CAF functions remains elusive. Single-cell technologies have allowed the identification of CAF subtypes with a differential impact on prognosis and response to therapy, but currently without clear consensus. Identification and pharmacological targeting of CAF subtypes associated with immunotherapy response offers new hope to expand clinical options for pancreatic cancer. Various CAF subtype markers may represent biomarkers for patient stratification, to obtain enhanced response with existing and emerging combinatorial therapeutic strategies. Thus, CAF subtyping is the next frontier in understanding and exploiting the tumour microenvironment for therapeutic benefit.

Keywords: cancer-associated fibroblasts; immunotherapy; in vivo models; knock-out models; myofibroblast; pancreatic cancer

Fibroblasts in cancer

All solid organs and, consequently all solid tumours, contain abundant populations of fibroblasts, making them key regulators of tumour biology. As in development, organogenesis, and wound healing, fibroblasts are critical for the establishment of tissue structure and integrity. Fibroblasts are the predominant source and regulators of the extracellular matrix (ECM). In turn, the ECM provides the structure necessary to support angiogenesis and the associated nutrient supply necessary to support organ function or tumour growth. In addition to providing tumour structure, numerous studies have implicated fibroblasts in the regulation of all aspects of tumour progression, including immune evasion, metabolic reprogramming, tissue invasion, and metastasis [1–3]. Cancer-associated fibroblasts (CAFs) differ from fibroblasts in healthy tissue, driven by complex reciprocal interaction with cancer cells [3–6]. Under the influence of the cancer microenvironment, CAFs can adopt a chronically activated alpha-smooth muscle actin (α-SMA)-expressing, contractile myofibroblast phenotype, comparable to the transient reversible phenotype adopted by fibroblasts in the wound-healing process. CAFs typically produce more ECM and ECM remodelling proteins, and have higher rates of proliferation than normal resident, and apparently quiescent fibroblasts [5]. In contrast to myofibroblasts involved in wound healing, CAFs may have limited ability to reacquire a quiescent state and can display resistance to apoptosis. CAFs are thus distinct from myofibroblasts acquired in acute and chronic inflammation [7].

Over recent years, a more complex picture has emerged as mesenchymal cell markers have been better defined, fuelled by the development of single-cell transcriptomic and proteomic technologies. This has revealed dramatic CAF heterogeneity, with distinct subpopulations playing diverse and often conflicting roles in the regulation of tumour biology. In addition to the classically recognised α-SMA^high myofibroblast phenotype CAFs, a range of CAF subsets associated with immune modulation have been identified. Distinct CAF
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populations show variable expression of classical fibroblast markers such as α-SMA, fibroblast activation protein (FAP), and podoplanin (PDPN). Some CAF subtypes or phenotypes appear to be interconvertible, while others appear restricted to distinct lineages. This necessitates a reappraisal of the source of distinct CAF subtypes and their evolving roles as tumours develop and respond to treatment.

Conflicting results from early attempts to target CAFs therapeutically

Numerous studies report that CAFs promote cancer cell growth, survival, invasion, and drug resistance. Elevated α-SMA expression is causatively associated with enhanced contractility, which can promote migration and tissue invasion [8,9]. Paracrine production of growth factors and cytokines, including hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and stromal-derived factor (SDF-1/CXCL12) support a protumourigenic, chemotherapy-resistant and immune-suppressive environment in a variety of cancers [6,10–16]. Pancreatic cancer models provide the most exhaustive preclinical rationale for the central role for CAFs in tumour biology. Supporting a role in metastasis, CAFs have been shown to directly lead cancer cell invasion by generating tracks through the ECM for cancer cells to collectively migrate and invade [17]. Further, the identification of pancreatic stellate cells (PSCs) at secondary metastatic sites in implantation murine pancreatic ductal adenocarcinoma (PDAC) models with gender mismatch implicates myofibroblasts directly in the distant spread of disease [18]. In PDAC, the initial indication that targeting stromal myofibroblasts might improve therapy response came from transgenic mouse models where inhibition of hedgehog signalling with IPI-926 depleted the α-SMA+ stroma to enhance vascularisation and gemcitabine penetration (perfusion) and, thus, response [19]. A variety of agents limiting CAF or PSC function in diverse preclinical models have now been shown to improve gemcitabine or immunotherapy responses for PDAC, including FAK inhibitors (VS-4718), vitamin A analogues (all trans-retinoic acid), and vitamin D receptor agonists (calcipotriol) [20–24].

In stark contrast to these promising results, efforts to suppress pathological CAF functions, by depleting α-SMA-positive fibroblasts or prevent stromal activation by targeting hedgehog signalling in multiple transgenic murine models of PDAC resulted in more aggressive, faster-progressing metastatic disease [25–27]. Furthermore, rather disappointingly, a Phase Ib/II clinical trial using hedgehog inhibitors (IPI-926) to block fibroblasts activation in combination with gemcitabine alone in pancreatic cancer patients was also terminated early due to disease acceleration (NCT01130142) [28]. Studies have additionally shown that impeding myofibroblast differentiation or functional phenotype in transgenic and implantation mouse models can result in more invasive inflammatory tumours [29,30]. Taken together, these data imply that the initial induction of myofibroblast CAFs (myCAFs) in response to malignant lesions may represent a tumour-suppressive response to limit cancer development through a variety of mechanisms. However, as tumours evolve this restraining role may be subverted to support invasion and metastasis. Hence, the timing and approach to modulate CAF behaviour as well as understanding how distinct subpopulations of CAFs contribute to favourable or unfavourable behaviour is of critical importance. Furthermore, understanding how these CAF subpopulations evolve with tumour progression and in response to treatment will be critical if we are to intervene clinically with success.

The ability of resident tissue fibroblasts to suppress malignant growth has long been established, and efforts to reprogram activated CAFs to their preactive quiescent state has shown sufficient promise to support clinical trials [21,23,31,32](NCT03520790). Treatment with vitamin A analogues or vitamin D receptor agonists to promote PSC quiescence, based on their physiological responsiveness to these nutritional stores, can suppress oncogenic signalling, tumour growth, and enhance chemotherapy response [21,24,33,34]. Linked to this, distinct subpopulations of patient-derived CAFs have been associated with differential prognosis, defined by their gene-expression levels [35]. In a transgenic K-Ras\(^{G_{12D}}\);LSL\(\text{G12D}_{\text{geo}}\);Trp53\(^{fl\text{-foxbox}}\);Elas\(-\text{TAI}	ext{AlteO-Cre}\) (KPeC) mouse model, a subset of Saa3 (Serum amyloid A apolipoprotein family) null CAFs can suppress cancer growth [36]. Using a combination of human samples and orthotopic KPC (Pdx1-Cre;Kras\(^{LSL-G_{12D}^{>>}}\);p53\(^{fl\text{-foxbox}}\)) derived murine models, as well as lineage tracing, a subpopulation of tumour restraining CAFs expressing Meffin (mesenchymal stromal cell- and fibroblast-expressing Linx paralogue; a glycosylphosphatidylinositol-anchored protein) have also been described in PDAC [37], which appear to cause alterations to collagen matrix layout. Lineage tracing also suggested that reprogramming of Meffin-positive PSCs or CAFs to α-SMA\(^{\text{high}}\) CAFs that are both positive and negative for Meffin expression may contribute to CAF functional heterogeneity in tumours. Screening for chemicals capable of promoting conversion of tumour promoting CAFs to rCAF identified the synthetic retinoid Am80 (Tamibarotene) as a promising candidate. Am80 upregulates Meffin expression in stromal cells and enhances gemcitabine chemotherapy response in a subcutaneous mT5 (KPC-derived PDAC cell line) mouse PDAC model [38], and is now being trialled clinically in combination with gemcitabine and nab-paclitaxel (Phase III; NCT05064618) [39]. Together these diverse studies suggest subpopulations of CAFs can exhibit both tumour-suppressor and -promoter functions at distinct stages of disease development.

A number of mechanisms may contribute to CAF tumour-suppressive functions, including suppression of inflammation and deposition of tumour-restraining matrix [2,40–42]. For example, α-SMA+ myofibroblast-specific deletion of ColI\(\alpha\) using a dual recombinant FSE-Kras\(^{G_{12D}^{>>}}\);Trp53\(^{fl\text{-foxbox}}\);Pdx1-Flp (KKPP); α-SMA-Cre; R26\(^{\text{Dual}}\) transgenic mouse model resulted in CXCL5...
upregulation in cancer cells, leading to augmented recruitment of CD206 + Arg1 + myeloid-derived suppressor cells (MDSCs), which in turn suppressed CD8+ T cells, leading to aggressive tumours [43]. This could be at least partially reversed by combined targeting of CXCR2 and CCR2, demonstrating a critical role for collagen and CAFs in the orchestration of the tumour microenvironment (TME) [43]. It remains to be seen whether the inherent differences between tumour-promoting and tumour-suppressing CAFs is spatially restricted within the juxta-tumoral space as opposed to the pan-stromal space [44], as well as temporally regulated as the tumour evolves: an aspect which will be explored by emerging spatially resolved sc-RNAseq and lineage tracing.

CAF heterogeneity and plasticity

Dichotomous roles for fibroblasts in tumour development have long supported the premise that distinct subpopulations of CAFs may modulate tumours differentially [4]. The coexistence of diverse fibroblasts has also been long appreciated [45]. In 2011, Kiskowski et al elegantly demonstrated, for the first time, that mixed populations of transforming growth factor-beta (TGF-β) responsive and TGF-β nonresponsive stromal cells can drive prostate adenocarcinoma, whereas alone these stromal components only support benign or precancerous lesions [46].

Myofibroblast CAF and inflammatory CAF switching Öhlund et al took the CAF dichotomisation a step further by defining two key interconvertible spatially resolved subpopulations of myofibroblast CAF (myCAF) and inflammatory CAF (iCAF) in both KPC mouse and human pancreatic cancer (Figure 1) [47]. MyCAFs broadly represent a classical TGF-β activated subtype, expressing high levels of the classical CAF markers α-SMA and FAP, and are found proximal to malignant cells. In contrast, iCAFs are found distal to tumour cells within the stroma and display low α-SMA expression with upregulation of TGF-β and IL1 signalling to promote distinct aspects of tumour biology, including ECM signatures, immune infiltrate, and malignant cell phenotypes. Created with BioRender.com.
### Table 1. Cancer-associated fibroblast subtypes and markers.

| CAF subtypes | Selected gene/protein markers | Biological functions/notes | Cancer type/model | Study type(s) | References |
|--------------|-------------------------------|---------------------------|-------------------|--------------|-----------|
| **Pancreatic cancer** | | | | | |
| myCAFs | Acta2, Ctgf, Postn | TGF-activated | Human PDAC/KPC mice | RNAseq | [29,47,49] |
| iCAFs | Ile, Cxcl1, Ccl12, Pdgfra, Has1, H2-Ab1, [IMHC class II] Cd74 (CD74), Soa3 | I1-activated. Promote inflammation | KPC mice | sc-RNAseq | [50,68] |
| apCAFs | POSTN/POSTN | Poor outcome | Primary pancreatic CAFs | NanoString nCounter | [35] |
| Subtype A | MYH11/MYH11, ACTA2/ACTA2 | Intermediate outcome | Human PDAC/KPP mice | sc-RNAseq | [65] |
| Subtype B | Lrc15(+) | Tumour promoting myCAFs. TGF-β promoted phenotype. | Human PDAC/KPP mice | sc-RNAseq | [65] |
| Subtype C | Dpp4, Ly6c1, Pdgfra | Inflammatory CAFs. IL1 promoted | | | |
| Subtype D | Cndt105 expression | Tumour permissive CAFs. TGF-β response | KPC mice and multiple GEMM cancer models | | [64] |
| **Breast cancer** | | | | | |
| vCAFs | Vegfa, Nid2 | Vascular development/angiogenesis. Perivascular origin. | MMTV-PyMT mouse model | sc-RNAseq | [73] |
| mCAFs | Flo1n, Pdgfra Dcn, Von, Col14a1, Cxcl14 | Matrix production/fibrosis. From resident fibroblasts. Decrease during progression | | | |
| dCAF | Srg1 | Malignant cell EMT | Human PDAC and KPC/Meflin-KO mice | IHC/ISH | [37,51] |
| myCAFs | Acta2, Lrc15 | TGF-β activated myCAFs | Subcutaneous 4T1 Breast cancer model | sc-RNAseq | [62] |
| iCAFs | Ile, C3, Cndt105 expression | Inflammation and immune cell regulation/recruitment | Human primary breast cancer | FACs sorted sc-RNAseq | [48,54] |
| vCAFs | Vegfa, Acta2, Lrc15 | Vascular CAFs - Vascular development/angiogenesis | | | |
| iiCAFs | CD74(+) | Interferon licenced CAFs. Induced on TGF-β blockade | | | |
| myCAFs | Acta2, PDNP, FAP | TGF-β activated myCAFs | Primary breast tumours | | [53] |
| iCAFs | CD34, CXCL1,CXCL12, CXCL13 | Inflammation and immune cell regulation/recruitment | | | |
| CAF-S1 | FAP, FSP-1, ACTA2, CD29 | Subsets of CAF-S1 include myCAFs (ecm-myCAFs and TGF-β-myCAFs) and iCAFs. myCAFs immunosuppressive | | | |
| CAF-S2 and S3 | CD29 | Normal tissue fibroblast signature | | | |
| CAF-S4 | CD29, ACTA2 | Cancer-associated | | | |
| **Selected other cancers** | | | | | |
| myCAFs | ACTA2, HAS2 | myCAFs promote Has2/HA axis | Hepatic Stellate Cell Origin | Cholangiocarcinoma (murine and human ICC) | sc-RNAseq | [65] |
| iCAFs | HGF, cytokines, chemokines | iCAFs promote growth through HGF | Hepatic Stellate Cell Origin | | |
| mesoCAFs | ACTA2, COL1A1 | Mesothelial CAFs | | | |
| myCAFs | ACTA2, COL1A1, CDH12, IL6 and CXCL14 | Myofibroblasts | Gastric cancer | | |
| iCAFs | CXCL12, IL6 and CXCL14 | iCAFs regulate T-cells | | | |
| eCAFs (ECM) | MMP14, LOXL2, and POSTN | Proteins and regulators of ECM | | | |
multiple tumour types [29,49,52,64]. The inflammatory FAP$^{\text{high}}$ phenotype, while dominant at the early phases of tumour development, appears to gradually give way to a more myofibroblastic $\alpha$-SMA+ CD34$^{\text{neg}}$ phenotype that is contractile and produces a stiff collagen-rich matrix as the disease progresses in transgenic KPC and KPP murine models [42,65]. Mechanistically, TGF-$\beta$ downregulates IL1R1 expression and thus suppresses the more secretory iCAF phenotype [29], which likely contributes to the spatial resolution of these cell types in tumours, where iCAFs are comparatively distal to the TGF-$\beta$-producing malignant epithelium [47]. Typically, FAP$^{\text{high}}$ $\alpha$-SMA$^{\text{low}}$ iCAFs are associated with increased tumour progression [29,49,57].

Strikingly, uncoupling myofibroblast functionality can also result in a switch from a myCAF expression signature to an iCAF signature in KPC-derived syngeneic orthotopic pancreatic tumours [30]. Loss of the Rho-effector kinase protein kinase N2 (PKN2) from PSCs in vitro suppressed cell contractility and mechano-sensing, while promoting adoption of an iCAF-like matrisome and expression of the iCAF markers IL6 and LIF. In vivo, stromal deletion of PKN2 also resulted in a shift from myCAF to iCAF signatures in orthotopic murine tumours, accompanied by enhanced EMT and IL6-JAK-STAT3 signalling. This implies that the role for PKN2 in myofibroblast function delineated in PSCs is conserved in CAF populations in orthotopic tumours. Similarly, targeting FAK in a subset of FSP-positive CAFs would also suppress mechanotransduction-mediated myofibroblast activation and likewise result in more aggressive PDAC tumours, accompanied by enhanced inflammatory chemokine signalling and a switch in tumour metabolism towards malignant cell glycosylation [58]. These results tightly concur with the concept that CAFs exist in interconvertible states, but also highlight that targeting one pathological function can result in bias towards distinct CAF subpopulations, with unexpected and sometimes undesirable consequences.

Many functionally distinct subpopulations of CAFs continue to emerge

Sc-RNA data reveals abundant fibroblasts, endothelial cells, pericytes, mesothelial cells, and immune cells as major stromal cell types in pancreatic tumours [59,65]. Historically, a lack of clearly defined markers has hampered isolation and definition of CAFs and their various subtypes. Classical markers such as $\alpha$-SMA, vimentin, FAP, PDGFR$\alpha$, and PDPN [9,50,60–62] have been useful, but their high-level expression by other cell types, such as pericytes, and heterogeneous expression across fibroblast subpopulations can confound deconvolution. Single cell-RNA sequencing (sc-RNAseq) of total tumour cell populations, coupled with focussed sc-RNAseq of fibroblast-enriched fractions, has resolved this problem by defining highly discriminatory stromal cell signatures. Importantly, sc-RNAseq data can also be used to infer CAF subpopulation functions and heterocellular interactions. Lineage tracking and temporal analysis has revealed diverse cell origins, differentiation trajectories, and variable interconvertibility between CAF subtypes. Despite significant heterogeneity within tumours, functionally distinct CAF subtypes identified are significantly conserved across distinct cancer types (Table 1).

In pancreatic cancer, multiple sc-RNAseq studies have now mapped CAF populations in mouse models and human primary tumours. Sc-RNAseq studies by Tuveson’s group characterised iCAFs and myCAFs, and identified a novel class of MHC class II and CD74 expressing ‘antigen presenting’ CAFs in KPC mouse and human tumours; KPC-derived apCAFs were able to activate CD4+ T-cells in an antigen-specific manner, in keeping with a role in tumour immune surveillance, at least in the KPC murine model [66]. Although apCAFs appear related to myCAFs in the KPC model, a mesothelial origin for apCAFs has been also been proposed in single-cell studies [64,65] (See Box 1). Leucine-Rich Repeat Containing 15 (LRRC15) expression was identified by Dominguez et al as a defining feature of CAFs over normal tissue fibroblasts in both Pdx1$^{\text{Cre/LSL-Kras}^{G12D/+}-p16/p19\text{lox/lox}}$ (KPP) mice and human pancreatic cancer patients [65]. CD105, an auxiliary receptor within the TGF-$\beta$ signalling pathway [80] additionally defines a precursor fibroblast population found in normal tissue [65]. Elegant pseudo-time analyses suggest that CD105+ resident fibroblasts give rise to LRRC15$^{\text{high}}$ myCAFs as tumours become established and progress, whereas an alternative lineage of CD105$^{\text{neg}},$DPP4$^{+}$ resident fibroblasts give rise to iCAFs. CAFs derived from both lineages show high-level expression of Coll1a1 and Colla2 [65]. In contrast, analysis of human samples suggests that iCAFs and myCAFs can derive from a single CD105$^{+}$ lineage, polarised to IL1 or TGF-$\beta$ activated states, urging caution when extrapolating from mouse models [65]. Differences may also reflect the pan-pancreas tumour development in these transgenic mouse models, as opposed to solitary tumour focus in humans.

Following this theme, Hutton et al used a combination of mass cytometry and transcript analysis to identify CD105 as a key CAF lineage marker defining tumour suppressive CAFs (CD105$^{\text{neg}}$), which act by supporting antitumour immunity [64]. Intriguingly, KPC-derived CD105$^{\text{neg}}$ and CD105$^{\text{neg}}$ CAFs were not interconvertible, but both could adopt either an myCAF or iCAF phenotype, potentially indicating multiple origins for these phenotypic classifications [64,65]. In line with Dominguez et al, it is proposed that CD105$^{\text{neg}}$ and CD105$^{\text{neg}}$ CAFs derive from distinct spatially resolved precursor fibroblasts and provide evidence that CD105$^{\text{neg}}$ precursors may be related to mesothelial lineages. The contribution of distinct fibroblast lineages in human PDAC is a hot topic for further exploration (See Box 1).

Some consensus is beginning to emerge for the categorisation of functionally conserved CAF categories. Meta-analysis of human sc-RNAseq data from multiple cancer types defined six pan-CAF subtypes and
Box 1. CAF origins and lineages.

CAFs have been reported to originate from many sources, including resident fibroblast populations, mesenchymal stem cells (MSCs) and transdifferentiation of distinct stromal populations such as adipocytes, pericytes and mesothelial cells [reviewed in [1,3,63]]. Additionally, distinct CAF subtypes appear to be dynamic and interconvertible, epitomised by spatial and cytokine regulation of iCAF and myCAF state in many disease settings. In pancreatic cancer, the existence of noninterconvertible fibroblast subtypes such as CD105high and CD105low populations, both in tumours and the healthy pancreas, indicates that distinct lineages are likely to contribute to heterogeneity [64,65]. Interestingly, CD105high and CD105low fibroblast populations can be found in distinct localisations in the normal pancreas and gene expression suggests a developmental link between CD105high fibroblasts and mesothelial cells [64]. Dominguez et al provide additional evidence that iCAFs may largely derive from a CD105low resident population [65]. That study also provided evidence that CD74 and H2-Ab1-expressing CD105high fibroblasts-equivalent to the apCAFs defined by Elyada et al [66]-have a mesothelial origin, although the origins of apCAFs may be distinct in KPC and human PDAC [65,66]. Mesothelial to mesenchymal transition has been reported in other pathological tissue fibrosis [67].

Garcia et al reported distinct Gli1 and HoxB6 fibroblast lineages in the healthy mouse pancreas, which can both contribute significantly to KPC ([Ptf1aFlpO+/+;RrasGF12D+/+] and KPC ([Ptf1aFlpO+/+;RrasGF12D+/+;Tpr53FRT-STOP-FRT/+]-) driven tumour CAFs, with Gli1+ cells dominating [66]. These distinct resident-fibroblast lineages, alongside lineage tracking, challenge the concept that pancreatic CAFs are predominantly derived from PSCs [69-71]. In fact, recent lineage tracing studies from the Sherman group indicate that PSCs appear to contribute only a minor subpopulation of CAFs in both KPC mouse orthotopic and human PDAC tumours [71]. Non-PSC pancreatic fibroblasts can expand into abundant α-SMAhigh CAFs in PDAC. This study also identified unique nonredundant functions associated with CAFs from distinct origins [71].

In the PyMT-MMTV breast cancer model [72], distinct CAF populations also appeared to derive from distinct resident mesenchymal lineages. Both resident tissue fibroblasts and mesenchymal cells with a perivascular origin contribute to tumour CAFs, which vie for dominance as tumours progress [73]. These studies highlight diverse lineage origins even within resident mesenchymal populations, with the caveat that differences between primary human tumours and mouse models are likely to exist.

The importance of MSCs as a source of CAFs appears to vary considerably between tumour types. In adoptive transfer experiments, bone marrow-derived cells were shown to contribute up to 25% of fibroblasts in a large-T-driver model of pancreatic insulitis, as well as contributing significantly to myofibroblasts in many tissues [74-76]. MSC-derived CAFs in breast cancer are also functionally distinct from resident fibroblast-derived CAFs, showing no expression of PDGFRα and associating with worse prognosis [77]. Interestingly, in a study of secondary tumours arising in sex-mismatched bone-marrow transplant recipients, the majority of α-SMA+ CAFs were recipient-derived [78]. In colorectal cancer most CAFs appear to be derived from resident pericytial fibroblasts [79]. Similarly in pancreatic cancer, resident mesenchymal populations appear to contribute the majority of CAFs, despite distinct lineage sources [64,65,68,70]. Finally, EMT of malignant cancer cells also contributes to α-SMAhigh CAF-like populations in tumours [73]. Though these cells are not classically considered CAFs, they are certain to contribute significantly to ECM and immune regulation, in addition to the well-documented role of EMT in migration, invasion, and metastasis. As distinct origins translate into distinct tumour-regulating functions, it is perhaps unsurprising that targeting ubiquitous CAF regulators can lead to unpredictable outcomes.

Manipulating CAF populations to promote therapy response

Immune evasion is a key hallmark of cancer [86]. While initial efforts to target CAFs focused on improving associated markers equating to myCAFs, iCAFs (pan-iCAF and pan-iCAF-2), dCAFs (ECM producing), nCAFs (normal fibroblast-like signature), and pCAFs (proliferating) [81]. Signatures derived from these pan-CAFs had different prognostic power between distinct cancer types [81]. In addition to the widely described myCAF and iCAF subcategories, CAFs dedicated to ECM regulation and proliferating CAF signatures have also been widely identified in mouse and human datasets (Table 1) [35,52,56,73,82].

While detailed maps of the CAF landscape have been provided for mouse models and a small number of primary human PDAC tumours, less has been done to examine interpatient and intrapatient variability in CAF populations. Transcriptomic analysis of primary CAF isolates from PDAC patients defined at least four distinct CAF subgroups with differing expression of known fibroblast identifiers and with a distinct impact on patient prognosis. For example, enrichment with α-SMAhigh ECM+ myofibroblast CAFs (pCAFassigner subtypes B and D) was associated with a worse prognosis, while α-SMAlow immunomodulatory CAF (pCAFassigner subtype C) signatures predicted better outcome when interrogated across ICGC and TCGA datasets [35]. Lee et al also conducted sc-RNAseq on primary tumour extracts, including metastases to identify tumour subtypes and heterogeneous TME responses and CAF content, which identified potential immunotherapy vulnerabilities [83]; high apCAF abundance relative to other CAF subtypes, was associated with low Teffector/TRegulatory (Teff/Treg) ratios, supporting a key role for CAF ratios in regulation of antitumour immunity. This study also highlighted the existence of multiple epithelial cancer subtypes within individual tumours, and metastases, independent of the classification system used [83]. Taking this a step further, Grünewald et al use spatially resolved multi-omics to powerfully demonstrate that CAF differentiation trajectories in subTME regions of PDAC tumours dictate localised tumour immunity, cancer cell phenotype, and treatment susceptibility [84]. Similar phenotypically distinct ‘tumour glands’ exhibiting distinct CAF-cancer cell relationships have also been reported by Ligorio et al [85]. Both studies highlight localised and heterogeneous intratumoral evolution of cancer-CAF relationships in primary human PDAC.

In summary, resident fibroblasts from distinct lineages most likely contribute to functionally distinct CAF populations as tumours evolve, with early subtypes (such as pCAFassigner subtype A [35]) being more pliable and later subtypes being more resistant to interconvertibility. Some CAFs may arise as a cause rather than a consequence of PDAC evolution [84,85] (See Box 1). In the next sections we will address the integral role that fibroblasts play in immune cell recruitment and whether CAFs can be manipulated to support specific therapeutic interventions.
chemotherapy responses, opening up tumours to the immune system has now become a key focus (Figure 2). CAFs play a key role in the recruitment and maintenance of immune cells in solid tumours, and are considered the architects of the immune suppressive environment. Importantly, immune engagement can improve the prospects for immunotherapy and chemotherapy responses, and support enduring antitumour protection.

CAFs can bias T-cell responses through a variety of mechanisms, including: (1) exclusion of cytotoxic CD8+ T-cells [42,44,87,88] by spatially restricting them to pan-stromal space as observed in human samples, and (2) promotion of T-regulatory cells as seen in murine models with interference of hedgehog signalling [29,49]. Evolution of cancer from precursor lesions to invasive disease is associated with progressive loss of effector T-cells and enhanced MDSC content, choreographed by progressive immunomodulatory CAF evolution [89]. However, Pancreatic intraepithelial neoplasia (PanIN) resolution in humans cannot and has not been studied to lend credence to the hypothesis that stromal- and immune-modulation is an active program. The key determinants of immune evasion are the presence of immune-suppressive cytokines and recruitment of immune-suppressive myeloid cells, biasing the immune response towards a more regulatory phenotype. Sc-CAF mouse studies have consistently shown CAF production of immunomodulatory cytokines such as Il6, Cyr61, and Cox-2, which can create immune suppressive environments that diminish the activity of effector immune cells. CAF-derived chemokines like Cxcl1, Cxcl12, and Cxcl2 also recruit a heterogeneous population of largely immunosuppressive or tumour-promoting myeloid-derived cells, including monocytes, macrophages, neutrophils, and MDSCs, at least in murine studies [41,42,65,89,90].

The unfavourable immunosuppressive impact of myeloid and lymphocyte immune cell infiltration on tumour growth may nonetheless be manipulated towards a favourable response to immunotherapy (Figure 2). Inducing a stronger inflammatory CAF phenotype in tumours has improved the response to immunotherapy and/or chemotherapy in murine models [25,42,65]. This provides evidence that manipulating distinct subpopulations of CAFs might hold the key to engaging immunotherapy in pancreatic and other solid cancers. Enhanced tumour inflammation has however been associated with more aggressive tumour growth in mouse PDAC models and worse prognosis in human datasets, and should be cautiously approached [29,30,40,91–94]. A balance of interventions must be found to concomitantly impede tumour progression, promote antitumour immunity, and enhance therapy efficacy.

Figure 2. CAF modulation of the immune microenvironment and immunotherapy. Different CAF subtypes have distinct tumour-promoting and tumour-suppressor functions. myCAFs can have both tumour-restraining but also support an immune-suppressive microenvironment that can block immunotherapy response. iCAFs produce inflammatory mediators and chemokines that can drive aggressive tumours with high EMT gene-expression signatures. While iCAFs can also support immunosuppression, enhanced inflammation and immune cell recruitment can also support enhanced immunotherapy response. Created with BioRender.com.
Targeting the immune suppressor CAF function

Evidence that CAFs regulate antitumour immunity was provided by Kraman et al., who showed that ablation of FAP+ stromal cells promoted tumour-antigen-specific immune clearance of lung and pancreatic cancer murine models [2]; this has since been confirmed in a murine KPC PDAC model [95]. More recently, Özdemir et al. demonstrated that targeted depletion of immunosuppressive α-SMA+ CAFs in a genetic PDAC model (Pkt: Ptf1aCrdt−/+; LSL-KrasG12D+/−; Tgβr1flox/flox) resulted in reduced fibrosis and accelerated tumour growth [25]; this was, however, accompanied by significant sensitisation to anti-CTLA-4 immunotherapy, with survival dramatically prolonged in comparison to non-CAF-depleted controls. This sensitisation was associated with enhanced Teff/Treg ratios and CTLA-4 expression and exemplifies the coexistence of protumoural roles with promotion of therapy response.

The CXCL12/CXCR4 axis also shows significant promise as a mechanism for reducing CAF-mediated fibrosis and enhancing checkpoint inhibitor response in both KPC pancreatic and orthotopic breast cancer murine models (Figure 2) [42,96]. This is also demonstrable in human samples with activated PSCs orchestrating this signalling [44], and this axis is now being targeted in clinical trials (NCT04177810; NCT02907099). In KPC mice, the inhibition of focal adhesion kinase (FAK) also reduces tumour fibrosis and immunosuppressive cell infiltration (MDSCs, tumour-associated macrophages and Treg) resulting in enhanced response to checkpoint blockade (anti-PD1 and anti-CTLA4) and chemotherapy [22]; in this model, tumour-cell intrinsic FAK appears to be the key driver of CAF expansion, tumour fibrosis, and immune suppression, in contrast to the previously described CAF intrinsic mechanisms. Stromal normalisation with the vitamin A analogue ATRA can enhance CD8+ T-cell recruitment to PDAC tumours and clinical trials are underway in combination with chemotherapy [23,44]. The impact of ATRA on checkpoint blockade remains to be assessed. In contrast, in vitro evidence might suggest that vitamin D agonists could suppress T-cell responses, although in vivo validation and combination with checkpoint inhibitors is currently lacking [97].

Hedgehog (Hh), an overexpressed protein in pancreatic cancers, appeared to be a promising target for treatment [19]. Despite early preclinical promise, targeting the hedgehog pathway inhibition with vismodegib has failed to improve chemotherapy responses in early clinical trials [98,99]. Surprisingly, although Hedgehog pathway inhibition has been trialled with additional targeted therapies against mammalian target of rapamycin (mTOR) or epidermal growth factor receptor (EGFR) pathways [100,101], combinations of hedgehog targeting with immunotherapy has been limited; a single clinical trial has recently begun combining the hedgehog pathway inhibitor NLM-001 with zalifrelimab (anti-CTLA-4) and chemotherapy (NCT04827953). In support of this approach, Patched 1-interacting peptide, which inhibits hedgehog signalling, reduced fibrosis, enhanced CD8+ T-cell infiltration, and augmented anti-PD1 response in mice [102]. Cotargeting the hedgehog pathway alongside CXCR4 also improved gemcitabine response in an orthotopic pancreatic model, which may be linked to CXCR4 regulation of antitumour immunity [42,96,103]. Further exploration of the hedgehog pathway in combination with immunotherapy is certainly warranted.

The immunosuppressive role of TGF-β has been examined in a variety of solid tumours, including PDAC [52,65,104,105]. TGF-β appears to drive an immunosuppressive myCAF landscape, with a poor response to checkpoint blockade. The response to anti-PDL1 treatment has been found to be diminished in human tumours across multiple cancer types enriched with TGF-β-driven LRRC15+ myCAFs [65]. Likewise, in metastatic urothelial cancers, the lack of response to anti-PD-L1 therapy was strongly associated with a TGF-β gene expression signature, indicating a possible role of fibroblasts in therapy resistance by sequestering CD8+ T cells in collagen and fibronectin-rich peritumoral stroma in patient samples. Furthermore, anti-TGF-β and anti-PD-L1 combination to provoke antitumour immunity and tumour regression in a mouse EMT6 mammary carcinoma model [104]. In concurrence, TGF-β neutralization in a subcutaneous 4T1 implantation model of breast cancer led to diminished myCAFs, enhanced CD8+ T-cell infiltration, and augmented anti-PD1 response [32]. Enhanced response was associated with an increase in iCAFs and the emergence of a CD73+ IFN-γ responsive CAF subtype (interferon-licenced CAFs, iCAFs); it currently remains unclear whether the increase in iCAFs and iCAFs results from reprogramming of existing myCAFs, or through expansion of distinct mesenchymal lineages. Switching of myCAFs to iCAFs in vitro provides support for conversion of existing myCAFs populations, although lineage tracing will be required to definitively answer this question [29,30,47]. In multiple genetic murine models of colorectal cancer, TGF-β inhibition also induced a potent antitumour immune response and enhanced anti-PD1-PDL1 therapy [105].

Kieffer et al. further defined specific subsets of FAP+ myCAFs in primary breast cancer responsible for immune suppression through association with enhanced FoxP3+ PD1+ Treg cells; importantly, in vitro coculture experiments with T-cells indicate that CAFs must adopt a myCAF phenotype to induce FoxP3, PD1 and CTLA-4 expression [48,54]. myCAF subtype but not iCAF subtype signatures are enriched in nonresponder groups in immunotherapy trial data for melanoma and non-small cell lung cancer (NSCLC), implicating myCAF-mediated immunosuppression in therapy evasion [48]. These studies make a case for the suppression of myCAF signatures to reduce immune evasion and improve immunotherapy response.

Are iCAFs desirable or dangerous in therapeutic strategies?

While there is some consensus on the existence of immunosuppressive myCAF populations, the role of iCAF...
subsets in immune suppression remains less clear. Biffi et al identified IL1-driven JAK-STAT3 as a key pathway governing iCAF identity, and further demonstrated that IL1 signalling antagonises TGF-β induction of myCAFs, resulting in the distinct polarised CAF clusters [29]. Importantly, the relative levels of iCAFs and myCAFs in KPC tumours can be modulated by targeting these pathways to assess the impact on tumour biology. Targeting the TGF-β pathway suppresses myCAFs and enhances iCAF populations, and is associated with more aggressive tumour growth, elevated inflammatory signalling, and EMT [29,49]. Conversely, targeting JAK or LIF to enhance myCAF populations was associated with less aggressive tumour growth [29,93]. Perhaps significantly, pharmacological targeting of JAK (JAKi) was associated with both enhanced myCAF/iCAF ratios and enhanced absolute CAF and myCAF numbers [29]; each of these changes may contribute to observed tumour phenotypes. More broadly, LIF and IL6 (key markers and regulators of iCAFs) have been variously associated with aggressive, inflammatory, EMT-rich poor outcome tumours [40,91–94]. This is further corroborated in human tumour data, where inflammatory, EMT, and iCAF signatures are all associated with poor outcome [29,49]. Similar phenotypic changes can be seen upon induction of an myCAF to iCAF switch by targeting the Rho-regulated kinase PKN2 to uncouple myofibroblast mechanotransduction [30]. Targeting IL1 signalling to limit iCAFs, has therefore been proposed as a method to suppress aggressive tumour growth [29]. Depletion of iCAFs could provide a therapeutic means to suppress production of tumour-promoting cytokines and chemokines while promoting the adoption of tumour suppressor myCAFs [29]. In opposition to this, targeting the hedgehog pathway with LDE225 shifts the balance away from myCAFs towards iCAFs and suppresses tumour growth [49]. This approach is at odds with the convincing identification of myCAFs as key immunosuppressive populations that block immunotherapy. Such studies would instead favour the suppression of myCAFs in favour of iCAFs to support improved immunotherapy response (Figure 1) [52].

iCAFs, by their definition, remain key mediators of the immune landscape in tumours. Enhanced myeloid cell content, skewing of Treg/Teff ratios, and loss of CD8+ cytotoxic T-cells have all been associated with iCAF-enriched tumour models, indicating potential immune suppressive roles [29,49]; here, a combination with appropriate checkpoint blockade may be of value. A PDPN+ immunofibroblast population has been directly associated with formation of tertiary lymphoid structures (TLS) dependent on IL13 and IL22 [106]. PDPN+ pCAFassigner subtype C CAFs have been shown to have an association with good prognosis and an immune-rich phenotype in human pancreatic cancers [35]. This is important because TLS content and activation status represent prognostic biomarkers for a good outcome and predictive biomarkers for response to immunotherapy in multiple tumour types, including PDAC [107–114]. In melanoma, PDPN+ CAF networks act as lymphoid organisers through production of TLS-promoting chemokines and through direct interaction with B cells, to orchestrate antitumour immunity [115,116]. Further, direct induction of TLSs in an orthotopic KPC pancreatic cancer model by intratumoural injection of CXCL13 and CCL21 has been shown to directly augment chemotherapy response [117]. While myCAF populations harbour key tumour-suppressor populations, iCAFs are likely to remain important regulators of leucocyte content and antitumour immunity. This complicated picture with conflicting roles for iCAFs and myCAFs reflects heterogeneity within these broad CAF categories, evolving roles during disease progression and inherent differences between tissues and CAFs from distinct origins.

Perspectives on clinical translation

Success in preclinical models has supported numerous clinical trials combining stromal targeting with established interventions in PDAC and other tumour settings (Table 2). In Phase Ib trials combining ATRA with gemcitabine and nab-paclitaxel, diffusion-weighted magnetic resonance imaging (MRI) has provided evidence that ATRA can effectively drive stromal modulation, and stromal expression of FABP5 has been identified as a potential predictive biomarker of disease response [23]. Randomised Phase II trials are underway (NCT03307148). A number of trials targeting the vitamin D receptor on PSCs with paricalcitol or high-dose vitamin D are also in progress, although initial results suggest no improvement in response rate or survival outcomes [119,120]. Some limited success, however, has been reported with targeting of the TGF-β axis in a variety of combinatorial studies. A combination of the TGF-β receptor I kinase inhibitor galunisertib with gemcitabine in a Phase I/IIb trial for unresectable pancreatic cancer resulted in improved patient survival [128]. Galunisertib trials with the anti-PD-L1 antibody durvalumab are also ongoing [121]. The novel bifunctional anti-PD-L1/TGF-βRII targeting fusion protein, SHR-1701, has also shown early promise in refractory solid tumours, including pancreatic cancer [122,124]. Despite a wide array of trials, targeting the hedgehog pathway has been largely unsuccessful. Vismodegib did not improve the outcome with either gemcitabine or gemcitabine and nab-paclitaxel. A combination of the hedgehog inhibitor IPI-926 with gemcitabine was also discontinued early due to poor results (NCT01130142) [28]. Targeting hyaluron directly in the pancreatic cancer stroma has also been the focus of significant clinical activity and some significant success, but this lies beyond the scope of this CAF-focussed review [133–137].

Pertinent to mixed results from trials, studies delineating the impact of stromal targeting have often relied on preclinical mouse models. In many cases tissue-specific Cre-lox conditional targeting is used to delineate the importance of pathways in specific stromal cell types.
Table 2. Clinical trials targeting stroma and CAF-related pathways in PDAC.

| Drug/trial name                                                                                                                                                                                                 | Years          | Phase | Target(s)                                                                 | Outcome and associated publications                                                                                                                                                                                                 | Type of cancer                                      | Trial ID and references |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|------------------------|
| ATRA in combination with gemcitabine and nab-paclitaxel (STAR-PAC) trial                                                                                                                                       | 2017–2020      | Ib    | Stroma, particularly stellate cells                                       | • Repurposing ATRA for stromal-targeting in combination with gemcitabine-nab-paclitaxel is safe and tolerable. This combination will be evaluated further in a phase II RCT for locally advanced PDAC.                                                   | PDAC                                               | NCT03307148            |
| Paricalcitol + gemcitabine + nab-paclitaxel                                                                                                                                                                    | 2018–2020      | I, II | Vitamin D receptors on pancreatic stellate cells                          | • Primary endpoint is overall survival with 100 patients needed to identify a HR of 0.6.                                                                                  | Metastatic pancreatic cancer                       | NCT03520790            |
| Paricalcitol in combination with paclitaxel, cisplatin, gemcitabine                                                                                                                                              | 2018–2023      | II    | Vitamin D receptors on pancreatic stellate cells                          | • Primary endpoint: complete response rate the end of cycle 3.                                                                                                             | Metastatic PDAC                                    | NCT0415854             |
| Pembrolizumab with or without paricalcitol                                                                                                                                                                     | 2017–2020      | II    | Vitamin D receptors on pancreatic stellate cells                          | • The primary endpoint is the percentage of people progressing at 6 months while on maintenance therapy.                                                             | Stage IV pancreatic cancer                        | NCT0331562             |
| Paricalcitol in combination with gemcitabine and nab-paclitaxel                                                                                                                                                | 2020–2024      | II    | Vitamin D receptors on pancreatic stellate cells                          | • Primary endpoint is PFS at 24 weeks from registration and OS and 18 months post last patient registration.                                                                | Advanced pancreatic cancer                        | NCT04617067            |
| Neoadjuvant paricalcitol (single agent)                                                                                                                                                                         | 2017–2021      | I     | Vitamin D receptors on pancreatic stellate cells                          | • Active, not recruiting                                                                                                                                                    | Resectable pancreatic cancer                      | NCT03300921            |
| Paricalcitol and nivolumab plus gemcitabine and nab-paclitaxel                                                                                                                                                 | 2020–2024      | Early phase I | Vitamin D receptors on pancreatic stellate cells                          | • Active, not recruiting                                                                                                                                                    | Resectable pancreatic cancer                      | NCT03519308            |
| Paricalcitol in combination with 5-FU/leucovorin plus liposomal irinotecan                                                                                                                                       | 2019–2022      | I     | Vitamin D receptors on pancreatic stellate cells                          | • Active, not recruiting                                                                                                                                                    | Advanced pancreatic cancer that progressed on Gemcitabine | NCT03883919          |
| Paricalcitol and hydroxychloroquine in combination with gemcitabine and nab-paclitaxel                                                                                                                                              | 2020–2023      | II    | Vitamin D receptors on pancreatic stellate cells                          | • Primary outcome measures: change from baseline tumour size on cross sectional imaging at 8 weeks. (every 8 weeks)                                                     | Advanced or metastatic pancreatic cancer           | NCT04524702            |
| Paricalcitol in combination with abraxane/gemcitabine                                                                                                                                                           | 2014–2020      | I     | Vitamin D receptors on pancreatic stellate cells                          | • Primary endpoint: number of adverse events.                                                                                                                                | Resectable pancreatic cancer                      | NCT02030860            |
| High-dose Vitamin D (single agent)                                                                                                                                                                             | 2018–2021      | III   | Vitamin D receptors on pancreatic stellate cells                          | • Terminated due to COVID-19 pandemic.                                                                                                                                       | Pancreatic cancer                                 | NCT03472833            |

(Continues)
| Drug/trial name | Years | Phase | Target(s) | Outcome and associated publications | Type of cancer | Trial ID and references |
|----------------|-------|-------|-----------|--------------------------------------|---------------|------------------------|
| **TGF-β targeting**<br>**TGF-β immunotherapy combinations**<br>Galunisertib + durvalumab | 2016–2019 | Ib | TGF-β receptor + Anti-PD-L1 antibody | Clinical activity was limited, but both drugs were well tolerated in 32 patients. Studying this combination in patients in an earlier line of treatment was suggested. Completed. | Metastatic pancreatic cancer: refractory, previously treated with ≤ 2 regimens (2nd or 3rd line) | NCT02734160 [121] |
| SHR-1701 | 2020–2022 | Ib, II | Bifunctional PD-L1 and TGF-β | Ongoing (Active, not recruiting) | Advanced Metastatic pancreatic Cancer | NCT0462417 |
| SHR-1701 | 2018–2021 | I | Bifunctional PD-L1 and TGF-β | SHR-1701 showed good safety and tolerability profile with promising antitumour activity in refractory solid tumours | Advanced solid tumours including PDAC | NCT03710265 [122] |
| NIS793 (with and without spartalizumab in combination with gemcitabine/nab-paclitaxel) versus gemcitabine/nab-paclitaxel alone | 2020–2022 | II | TGF-β PD-L1 | Ongoing (Recruiting) | Metastatic PDAC | NCT04390763 [123] |
| SHR-1701 | 2020–2021 | II | Bifunctional PD-L1 and TGF-β | The combination showed promising activity with well-tolerated toxicities in patients with advanced pancreatic and biliary tract cancers | Previously treated advanced pancreatic and biliary cancers | ChiCTR2000037927 |
| NIS793 + spartalizumab (PDR001) | 2017–2021 | Ib | TGF-β PD-1 | Completed | Multiple including PDAC | NCT02947165 [125] |
| **TGF-β alone or with chemotherapy**<br>LY2157299 (galunisertib) in combination with gemcitabine | 2014–2015 | Ib | TGF-βR1 | Completed | Metastatic or locally advanced pancreatic cancer. | NCT01373164 [127] |
| Trabedersen (AP12009) (single agent) | 2005–2011 | I | TGF-β2 | Completed | Pancreatic cancer | NCT00844064 [126] |
| NIS793 in combination with gemcitabine/nab-paclitaxel versus gemcitabine/nab-paclitaxel and placebo | 2021–2022 | III | TGF-β | Ongoing (Recruiting) | Metastatic PDAC | NCT04935359 |
| Vactosertib (TEW-7197) in combination with FOLFAR | 2018–2019 | Ib, II | TGF-β/SMAD | Recruitment status: Unknown | Metastatic PDAC (refractory to gemcitabine and nab-paclitaxel) | NCT03668632 |
| LY2157299 (galunisertib) + gemcitabine | 2011–2016 | II | TGF-βR1 | Completed | Metastatic, unresectable pancreatic cancer | NCT01373164 [128] |
| ALK5 | | | | | | |

(Continues)
| Drug/trial name | Years | Phase | Target(s) | Outcome and associated publications | Type of cancer | Trial ID and references |
|----------------|-------|-------|-----------|------------------------------------|----------------|------------------------|
| PF-06952229 combination therapy with enzalutamide | 2018–2022 | I, Ib | TGF–βR1 | Active, not recruiting; Outcome not published | Advanced solid tumours [multiple including pancreas] | NCT03685591 |
| Hedgehog inhibitor combinations | | | | | | |
| NLM-001 in Combination with gemcitabine and nab-paclitaxel plus zalifrelimab. | 2021–2022 | I, II | Hedgehog pathway + CTLA-4 | Primary outcome measures: Objective response rate (ORR); Complete response (CR); Partial response (PR); Ongoing (Recruiting); Outcome not published | Advanced PDAC | NCT04827953 |
| LDE-225 (erismodegib) (single agent) | 2012–2014 | I | Hedgehog pathway | Trial withdrawn due to lack of accrual | Surgically resectable pancreatic cancer | NCT01694589 |
| LDE-225 (erismodegib) (single agent) | 2013–2016 | NA | Hedgehog pathway | Trial withdrawn (No accrual) | Surgically resectable pancreatic cancer | NCT01911416 |
| GDC-0449 (vismodegib) + gemcitabine | 2010–2017 | II | Hedgehog pathway | Primary outcome: Median percentage of CD44 + CD24 + ESA+ cells from FNAC at 3 weeks versus baseline. | Metastatic pancreatic cancer | NCT0195415 [98] |
| GDC-0449 (vismodegib) in combination with gemcitabine and nab-paclitaxel | 2010–2018 | II | Hedgehog pathway | Completed | Metastatic pancreatic cancer | NCT01088815 [99] |
| IPI-926 (saridegib) + gemcitabine | 2010–2017 | I, II | Hedgehog pathway | Completed | Metastatic pancreatic cancer | NCT01130142 [129] |
| LDE-225 (sonidegib) in combination with gemcitabine and nab-paclitaxel. (MATRIX trial) | 2015–2019 | I, II | Hedgehog pathway | Completed | Pancreatic cancer | NCT02358161 [130] |
| Vismodegib in combination with gemcitabine (NEOPACHI-001) | 2012 | I | Hedgehog pathway | Recruitment status: Unknown | Resectable PDAC | NCT01713218 |
| LDE-225 (sonidegib) in combination with fluorouracil, leucovorin, oxaliplatin, irinotecan | 2011–2020 | I | Hedgehog pathway | Completed; Outcome not published | Untreated advanced pancreatic cancer | NCT01485744 |
| GDC-0449 (vismodegib) and erlotinib with or without gemcitabine | 2009–2022 | II | Hedgehog pathway | Ongoing (Active, not recruiting); GDC-0449 and Erlotinib were tolerated at a dose of 150 mg each, and were suitable for evaluation at phase II | Metastatic or non-operable pancreas cancer | NCT00878163 |
| Drug/trial name | Years | Phase | Target(s) | Outcome and associated publications | Type of cancer | Trial ID and references |
|----------------|-------|-------|-----------|-------------------------------------|----------------|------------------------|
| GDC-0449 (vismodegib) in addition to gemcitabine | 2009–2013 | I, II | Hedgehog pathway | • Completed  
• Primary OM: PFS  
• Secondary OM: OS, ORR, Adverse events, Overall response rate  
• Addition of vismodegib to gemcitabine did not improve the overall response rate, PFS, DFS | Metastatic pancreas cancer | NCT01064622 [28] |
| LDE-225 (sonidegib) in combination with gemcitabine and nab-paclitaxel | 2011–2020 | I, II | Hedgehog pathway | • Study terminated (manufacturing of study drug ceased) | Borderline resectable pancreatic cancer | NCT01431794 |
| Other pathways | | | | | | |
| Am80 (tamborotene) [MIKE-1] | 2021–2025 | I, II | Meflin (Reprogram pCAF to rCAF) | • Primary OM: DLT (phase I), RR(phase II)  
• Secondary OM: AE, OS, PFS  
• Ongoing (Recruiting) | Unresectable PDAC | NCT06064618 [37,39,51] |
| Gemptinab + Nab-paclitaxel to target stroma | 2011–2013 | II | Stroma (Density) and tumour vessels and metabolism. | • Completed  
• Primary Outcome: effect on: stroma density, tumour vessel formation, tumour metabolism on PET-CT.  
• Secondary Outcome: activity against PDAC  
• Outcome not published | PDAC | NCT01442974 |
| Pamrevlumab + gemcitabine + nab-paclitaxel or pamrevlumab + FOLFRINOX | 2019–2023 | III | CTGF | • Active, not recruiting  
• Primary OM: OS, R0 and R1 resection achieved.  
• Secondary OM: EFS, PFS  
• Outcome not published | Locally advanced unresectable pancreatic cancer | NCT00941093 |
| Losartan + FOLFORINOX + proton beam radiation | 2013–2020 | II | Angiotensin receptor (targeting reduces collagen and hyaluronan levels) | • Active, not recruiting  
• Primary OM: R0 resection proportion.  
• Secondary OM: PFS, OS, toxicity, rate of downstaging, QoL  
• Combination resulted in downstaging of locally advanced PDAC, with associated R0 rate of 61% | Locally advanced PDAC | NCT01821729 [131] |
| Losartan and nivolumab in combination with FOLFRINOX and SBRT | 2021–2025 | II | Angiotensin receptor (targeting reduces collagen and hyaluronan levels) | • Active, not recruiting  
• Primary OM: R0 resection proportion.  
• Secondary OM: PFS, OS, Pathologic complete response, SAE  
• Outcome not published | Localised pancreatic cancer | NCT05632488 |
| Simtuzumab + gemcitabine | 2011–2015 | II | LOXL2 | • Completed  
• Primary OM: PFS  
• Secondary OM: OS, Objective response  
• Addition of simtuzumab to gemcitabine did not improve clinical outcomes | Metastatic PDAC | NCT01472198 [132] |
| Pembrolizumab without or without defactinib | 2019–2023 | II | FAK PD-L1 (Immunotherapy combination) | • Ongoing (recruiting)  
• Primary OM: Pathologic complete response  
• Secondary OM: OS, DFS, drug related toxicities  
• Outcome not published | Resectable PDAC | NCT03727880 |
on tumour biology. While informative, it is important to recognise that drug interventions will in most cases target malignant and stromal compartments, which can dramatically alter the outcome. As an example, targeting FAK genetically in FSP-positive CAFs resulted in metabolic switching of the malignant tumour, more aggressive growth, enhanced inflammatory chemokine signalling, and a switch in tumour metabolism [58]; FAK is a key regulator of myofibroblast function and this study concurs with other interventions suppressing myCAF function in PDAC tumours [25,26,29,30]. In contrast, targeting FAK systemically with kinase inhibitors in KPC mice targets both tumour and stroma, resulting in reduced fibrosis and tumour growth, and an enhanced response to both chemo- and immunotherapy [22]. Interestingly, the suppressive effect on CAFs and fibrosis is primarily driven by inhibition of FAK in tumour cells, to limit paracrine activation of the stroma.

Interestingly, a number of therapeutic approaches that suppress CAF functions appear to induce EMT signatures in malignant cells, with enhanced invasion and/or metastasis [29,30,93]. Counterintuitively, suppressing the contractile and invasive capacity of CAFs can promote more aggressive invasive behaviour of cancer cells. In the context of pharmacological intervention, it is noteworthy that pathways driving migration and invasion are likely to be shared by migratory cancer cells and fibroblasts, so drugs targeting myofibroblast-led invasion are also likely to impede tumour cell invasion. In a variety of mouse models, targeting Rho-associated kinase (ROCK) has been reported to block activation of CAFs, induce matrix remodelling, and also impede cancer-cell migration [138–142]; a dual impact on CAFs and cancer cells appears likely to contribute to the efficacy of these compounds. ROCK-targeting compounds used in these studies, including Y27632 and fasudil, also target the Rho-effector kinase PKN2, which also regulates migration and invasion of mesenchymal cancer cells and fibroblasts [30,143,144]. Many additional pathways involved in mesenchymal invasion are also likely to represent dual targets in both cancer and stroma, including Rho family members, integrins, FAK, and the mechanotransduction apparatus. As a broader lesson, genetically engineered mouse models that target specific compartments to understand the biological contribution of specific cell types to tumour biology, will not model the impact of targeting signalling cascade pharmacologically across all tumour compartments. This may lead to apparent contradictory results from genetic manipulation in transgenic models versus drug targeting with small molecules or antibodies, as well as in combinatorial approaches.

Chemotherapeutics or targeted therapies aimed at killing or suppressing cancer cell growth can also have a significant impact on the stroma. Erstad et al demonstrated that fibrosis associated with FOLIRINOX (oxaliplatin, 5-FU, leucovorin, and irinotecan) and radiation therapy predicts better patient outcome in pancreatic cancer [145]. Although pretreatment controls are lacking in that study, FOLFIRINOX also reduced tumour size and enhanced fibrosis in two murine syngeneic orthotopic models. Related to immunotherapy, the PARP inhibitor olaparib, used in the treatment of BRCA mutant cancers has been shown to have a beneficial impact on T-cell targeting by modulation of SDF1α (CCL12) production by CAFs [146]. In a less fortuitous example, targeting of BRAF mutant melanoma with the BRAF kinase inhibitor PLX4720 also drives activation of a fibrotic stromal response, which can result in therapy resistance [147]. Here the paradoxical activation of the Raf–ERK pathway in CAFs by PLX4720 drives integrin-FAK-mediated matrix production to protect the malignant epithelium. These studies highlight the importance of taking a holistic view of the impact of therapy on tumour biology. While mouse models can be invaluable in understanding the mechanism of action, most clinical therapies will target the malignant epithelium, stromal cells, immune infiltrate, and the systemic immune system, which can all impact therapy response.

Stromal roles for CAFs are context-specific

Sc-RNaseq of primary tumour biopsies has revealed the potential for therapeutic stratification based on detailed subtyping and stromal analysis [83], although this technology remains some distance from clinical application. A number of studies have classified PDAC into distinct subtypes, based largely on bulk transcriptomic data, with an impact on prognosis, therapy response, and tumour pathology [148–150]. In a landmark study, Moffitt et al [148] used a bioinformatic approach to virtually dissect tumours to identify distinct stromal signatures in PDAC from bulk RNAseq data. Importantly, this demonstrated that an activated ‘myofibroblast CAF-like’ stromal signature was independently associated with poor outcome. Importantly, however, the prognostic power of the stromal signature was also PDAC subtype-dependent, showing good prognostic power in classical-subtype PDAC but no power in basal-like PDAC [148]. It might be surmised that in basal-like PDAC, malignant cells may intrinsically exhibit more invasive characteristics and therefore the impact of invasive activated stromal CAFs may be diminished. With regard to mutation status, gain-of-function TP53 mutation has been shown to drive the generation of specific metastatic CAF populations, which can also protect cancer cells from therapy, at least in part through modulation of the matrisome [151]. Targeting the stroma to impact therapy response can thus be influenced by both mutational and the disease subtype context. Layered on to this, multiple disease subtypes coexist within individual tumours, in spatially resolved CAF-regulated microenvironments [83–85]. Successful targeting of CAF function must be tailored to both tumour and stromal signatures if response rates in trials are to be improved. Preclinical studies, where disease genetics are uniformly controlled, demonstrate the promise of stromal targeting.
but we cannot expect these to model the heterogeneity seen in advanced disease in patient populations.

The challenge is to identify which patients are likely to benefit from specific CAF/stromal targeted therapy, and in the context of which anticancer therapeutic strategies. Overlapping tumour-promoting and tumour-suppressing roles, coupled with a context dependence of stromal interventions, must be considered. In clinical trials, where heterogeneity, within the tumour, stroma, and the patient population, generates many variables, and the results have unsurprisingly been mixed, with no stromal therapies adopted in mainstream clinical practice. Furthermore, studying large cohorts of human cancer samples will enable better understanding of stromal heterogeneity. Currently, human primary CAF characterisation studies involve only a handful of patients, although the translational value is clear [35,83]. Progress will be critically supported by meta-analyses of existing trial data; in trials where response rates are poor, the focus now falls on identifying parameters linked to therapeutic responses. Identification of specific CAF subtypes and stromal signatures have been successful at identifying CAF signatures associated with immunotherapy response, and these can be brought to bear in the clinic [64,65,84]. Only this more informed evidence-based approach will improve the appropriate recruitment and success of clinical trials and subsequent tailoring of clinical pathways. Currently, initiatives of personalised medicine for anticancer treatment such as MSKCC-Impact [152], Precision-Panc [153] (NCT03770468), FOCUS-4 (NCT03770468), TRACERx, (NCT01888601) rely on genome or transcriptome analysis focusing on the tumour cell compartment. We envisage a future where the whole TME will be taken into account, whilst delivering anticancer treatment.

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Author contributions statement

SM and AJMC were responsible for the focus of the review and wrote the article. MHO was responsible for reviewing and summarising current and past clinical trials targeting CAFs in PDAC and other cancers, and critically edited the article. SMAJ was responsible for contributing to writing, and reviewed CAF heterogeneity in breast cancer. HMK was responsible for contributing to writing and critically editing the article to provide clinical context.

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