Context-Specific Determinants of the Immunosuppressive Tumor Microenvironment in Pancreatic Cancer

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ABSTRACT Immunotherapies have shown benefits across a range of human cancers, but not pancreatic ductal adenocarcinoma (PDAC). Recent evidence suggests that the immunosuppressive tumor microenvironment (TME) constitutes an important roadblock to their efficacy. The landscape of the TME differs substantially across PDAC subtypes, indicating context-specific principles of immunosuppression. In this review, we discuss how PDAC cells, the local TME, and systemic host and environmental factors drive immunosuppression in context. We argue that unraveling the mechanistic drivers of the context-specific modes of immunosuppression will open new possibilities to target PDAC more efficiently by using multimodal (immuno)therapeutic interventions.

Significance: Immunosuppression is an almost universal hallmark of pancreatic cancer, although this tumor entity is highly heterogeneous across its different subtypes and phenotypes. Here, we provide evidence that the diverse TME of pancreatic cancer is a central executor of various different context-dependent modes of immunosuppression, and discuss key challenges and novel opportunities to uncover, functionalize, and target the central drivers and functional nodes of immunosuppression for therapeutic exploitation.

INTRODUCTION

Despite significant advances in treating many tumor entities, therapeutic outcomes for patients with pancreatic ductal adenocarcinoma (PDAC) have remained almost unchanged over the years (1, 2). Due to an increasing incidence, late diagnosis, and lack of novel therapies, PDAC surpassed breast cancer in the last decade, becoming the third leading cause of cancer-related death in the Western world (2). Standard of care for most patients remains conventional cytotoxic polychemotherapy, with limited clinical success but high toxicity (1). This results in one of the highest death rates among all cancer types and a devastating 10-year overall survival of ~1% (1). With a persisting increase in incidence, PDAC is projected to become the second leading cause of cancer-related deaths in Western countries by 2030 (3), demonstrating a high unmet clinical need to develop new treatment strategies.

PDAC is genetically complex and characterized by diverse tumor microenvironments (TME), which influence disease prognosis and treatment outcomes. In contrast, immunosuppression is an overarching and almost universal hallmark of PDAC, even across the highly heterogeneous morphologic and molecular subtypes that have recently been defined. Current subtype classifications are based on morphologic features, such as the differentiation status of the tumor, or molecular characteristics, including genetic, epigenetic, transcriptional, and metabolic traits or combinations thereof. Importantly, the distinct categories identified so far reflect both tumor cell–intrinsic and microenvironment-specific aspects (4–14). However, immune cell populations are currently not taken into consideration in most subtyping studies. Based on the existing approaches, two main extreme subtypes of PDAC emerge: (i) classical tumors, composed of

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Cancer Discov 2023;13:278–97

doi: 10.1158/2159-8290.CD-22-0876

BOX 1: PDAC SUBTYPING STRATEGIES AND CLASSIFICATION.

Exploiting both genomic and transcriptomic profiling of surgically resected human PDAC, aided in some cases by components from the desmoplastic stroma, has shed light on the existence of distinct evolutionary routes toward PDAC, resulting in several, in part overlapping, subtypes (4–14). Classical tumors retain a gland-forming component, expression of endodermal lineage–specifying factors, such as GATA6, and are characterized by classic gene expression signatures. Mesenchymal tumors (also known as squamous, quasi-mesenchymal, or basal-like) are characterized by basal-like gene expression programs and the reduction of gland-forming structures characteristic of classical tumors. Recent reports have demonstrated that oncogenic $KRAS^{G12D}$ expression and copy-number variation have a dramatic effect in defining these subtypes. Indeed, mesenchymal PDAC shows the highest gene expression and increase in gene dosage of oncogenic *KRAS* (11, 95). Importantly, mesenchymal tumors are characterized by the worst overall survival and response to standard-of-care chemotherapy (7). These subtyping studies have been extensively reviewed elsewhere (4, 14).

cancer cells with glandular features, surrounded by abundant stroma with a classic epithelial gene expression signature and (ii) undifferentiated non–gland-forming tumors with less prevalent stroma and a basal-like gene expression program. Both subtypes have been shown to coexist in certain tumors (ref. 15; for more details see Box 1).

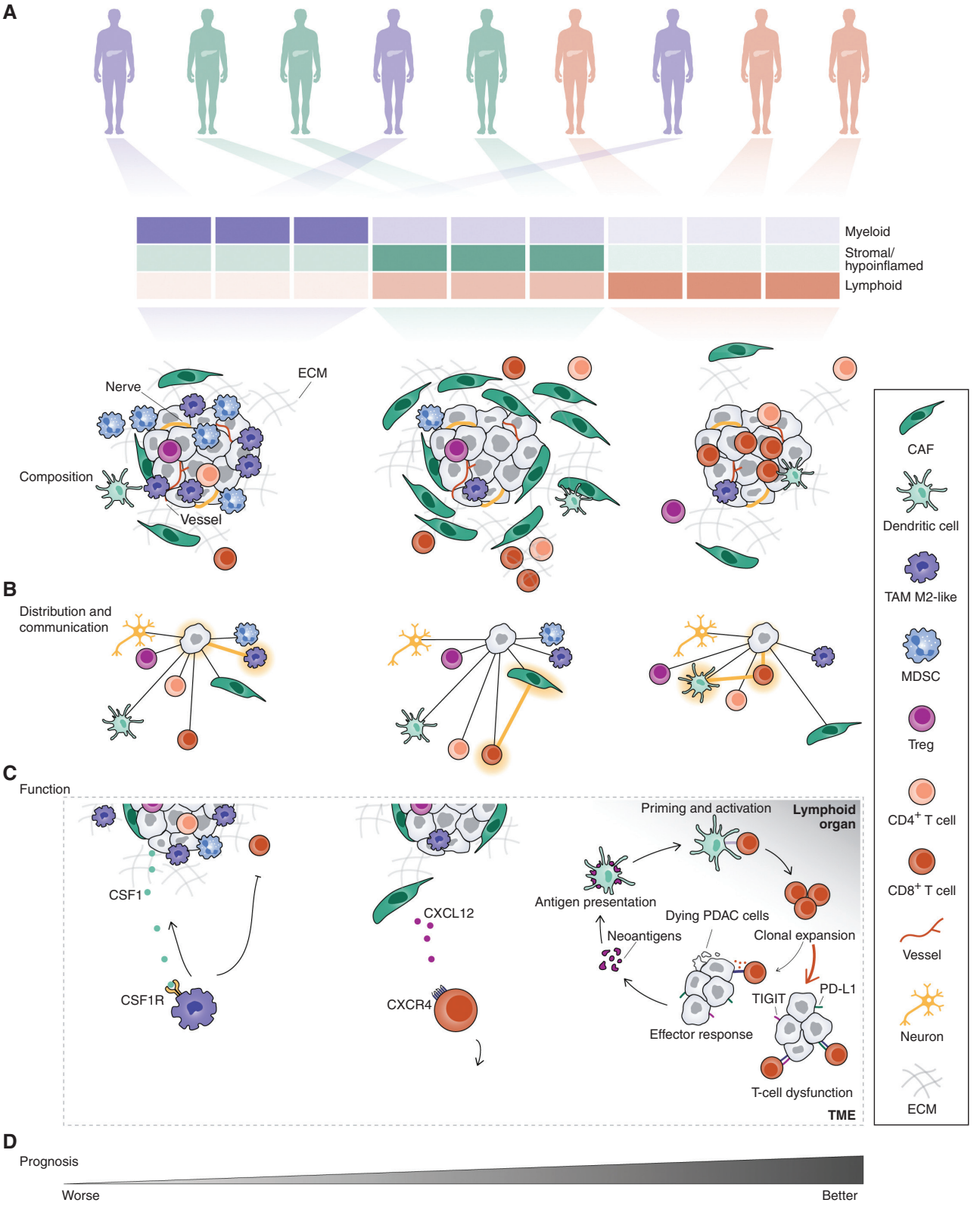
Despite clear differences in the composition of the TME, its immunosuppressive features and the driving mechanisms leading to distinct immune landscapes have not been systematically investigated yet. So far, different amounts of stromal and immune cell types have been associated with distinct prognosis in patients with PDAC (Fig. 1A–D). For instance, high levels of tumor-infiltrating CD3 T cells are predictive for longer progression-free survival (PFS; ref. 16). Moreover, different tumor cell differentiation states are associated with distinct stromal compositions (14). Therefore, merging both perspectives—that is, tumor cell–intrinsic and tumor microenvironment—is of fundamental importance to gain a holistic view of the disease and to inform novel therapeutic strategies.

Immune-checkpoint blockade (ICB) immunotherapy, targeting PD-1/PD-L1 or CTLA-4, has shown potential only in a small subset of patients with PDAC. Indeed, less than 1% of patients with PDAC, presenting with hypermutated microsatellite instable (MSI) tumors and demonstrating antigen-specific T-cell responses, have shown positive outcomes when treated with anti–PD-L1 ICB (17). More recently, homologous recombination–deficient (HRD) PDACs, which display higher mutational burden due to mutations in genes such as *BRCA1* and *BRCA2*, have been shown to benefit from ICB. In a retrospective, single-institution case study that tested the combination of ipilimumab (anti–CTLA-4 antibody) and nivolumab (anti–PD-1 antibody), 4 of 12 patients with HRD metastatic pancreatic or biliary cancer responded to this combination therapy. Responders showed higher tumor-infiltrating lymphocytes and higher expression of CCL4, CXCL9, and CXCL10 (18). In line, *BRCA1* and *BRCA2* mutations have been shown to positively correlate to PD-L1 staining in human PDAC (19). In addition, maintenance therapy with PARP/CTLA-4 double blockade was superior to PARP/PD-1 at the primary endpoint of 6 months in a phase Ib/II trial (NCT03404960) for patients with advanced pancreatic cancer whose cancer had not progressed for 16 weeks after platinum-based therapy (20). Even though these results hold promise for the identification of subsets of PDAC patients benefiting from immunotherapy, the responses and PFS rates observed so far are inferior to what has been shown for melanoma or non–small cell lung cancer, even in hypermutated MSI-high/DNA mismatch repair–deficient (dMMR) PDAC (21). Indeed, the response rates of MSI-high/dMMR PDACs are inferior to almost all other MSI-high/dMMR cancer types (21).

These data indicate that although the PDAC TME shows heterogeneity across distinct tumor subtypes, its unifying feature is immunosuppression. This raises the question: Why are PDAC subtypes uniformly resistant to immune clearance and how is immunosuppression achieved in context? Here, we will discuss evidence for the existence of several modes of immunosuppression and place them in a contextual framework of (i) tumor cell–intrinsic features of PDAC; (ii) non–tumor cell–autonomous characteristics shaped by the TME; and (iii) traits of the host, including genetic variation, injury, infection, and inflammation (i.e., pancreatitis), nutrition, obesity and metabolism, the microbiome, and environmental factors, such as toxins. First, we focus on key aspects and cell types composing and driving the context-specific immunosuppressive TME of PDAC. Then, we will highlight how interventions, including standard-of-care chemotherapy and targeted therapies, can alter the TME landscape and discuss how immunosuppression can be therapeutically targeted. Further, we will discuss fundamental questions and future multidisciplinary lines of research that should be pursued to elucidate the drivers of immunosuppression mechanistically. These efforts will guide the design of next-generation clinical trials and the implementation of personalized and/or stratified immunomodulatory therapies beyond checkpoint inhibition for patients with PDAC.

DIVERSITY OF THE PDAC TME LANDSCAPE

Classical PDAC cells are usually embedded in a diverse desmoplastic stroma, which is composed of extracellular matrix (ECM), cancer-associated fibroblasts (CAF), endothelial cells (EC) and pericytes, nerves, and different populations of immune cells (Fig. 1A). Immune cells include mostly myeloid cells, such as tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and neutrophils, but also T and B cells, dendritic cells (DC), and natural killer (NK) cells. Typically, PDAC's TME lacks active infiltrating $CD8^+$ T cells, which if present show low levels of activation markers such as GZMB and IFNG (22). Additional factors composing the TME include secreted molecules, such as growth factors,


A
Myeloid
Stromal/
hypoinflamed
Lymphoid
Nerve
ECM
Composition
Vessel
B
Distribution and
communication
C
Function
CSF1
CSF1R
CXCL12
CXCR4
Priming and activation
Lymphoid
organ
Antigen presentation
Dying PDAC cells
Neoantigens
Clonal expansion
PD-L1
TIGIT
Effector response
T-cell dysfunction
TME
CAF
Dendritic cell
TAM M2-like
MDSC
Treg
CD4+ T cell
CD8+ T cell
Vessel
Neuron
ECM
D
Prognosis
Worse
Better

cytokines, chemokines, and extracellular vesicles, but also the vascular network that participates in the complexity of this environment. Depending on the differentiation status of the tumor and a variety of other factors, the stroma can change dramatically showing sparse ECM deposition, differences in fibroblast activation and immune cell infiltration (Fig. 1A–C). Despite this heterogeneity in stromal composition and architecture, PDAC's TME is almost uniformly immunologically "cold" and strongly immunosuppressive—in many instances deserted of antitumor T cells (22).

Recent studies have focused on increasing our understanding of PDAC's TME heterogeneity via a comprehensive evaluation of the composition and distribution of stromal and immune elements. Using formalin-fixed, paraffin-embedded (FFPE) PDAC tissue sections and multiplexed imaging, groups of patients with different infiltration of immune cells were identified (23). Patients with PDAC showing the highest infiltration of $CD8^+$ and $CD4^+$ T cells displayed prolonged survival, and this was particularly true for $CD8^+$ T cells when localized in proximity to tumor cells (23). Moreover, tumor cells with high or low $CD8^+$ T-cell infiltration did not exhibit differences in stromal composition, as evaluated by α-smooth muscle actin (αSMA) and collagen I staining, suggesting that T-cell infiltration is independent from these stromal and ECM markers (23). The analysis of 1,824 tissue microarray specimens from 385 surgically resected patients included in the European Study Group for Pancreatic Cancer trials 1 and 2 by immunohistochemistry revealed distinct stromal signatures and heterogeneity with respect to tumor immune composition and prognostic relevance (16). The best postoperative PFS was observed in patients harboring a $CD3^{hi}CD206^{hi}$ signature, whereas patients with $CD3^{lo}CD8^{lo}CD68^{hi}$ showed the worst (16). Unbiased immune clustering of highly multiplexed immunofluorescence PDAC tissue imaging of 135 therapy-naïve and neoadjuvant-treated human PDACs revealed, independently of histopathologic annotation, three clusters based on their leukocyte profiles: (i) a myeloid-enriched, (ii) a lymphoid-enriched, and (iii) a hypoinflamed subgroup (Fig. 1A), which are in part linked to the molecular PDAC subtypes described above (see also Box 1). The lymphoid-enriched cluster showed a trend toward increased overall survival, corroborating previous findings. However, the resulting immune atlas also displayed great intra- and interpatient leukocyte heterogeneity, and protumoral infiltrates of suppressive myeloid cells and PD-1–negative T cells in all subgroups (24).

Tertiary lymphoid structures (TLS) add another layer of complexity and heterogeneity to the PDAC immune TME. They represent ectopic lymphoid aggregates that form in nonlymphoid tissues and are linked in many cancer types with a better prognosis and response to ICB (25). TLS have been identified in a subset of patients with PDAC, and their existence and abundance hold significant survival advantage with the best prognosis linked to a high density of B-ell aggregates (26–28).

Recently, single-cell RNA sequencing (scRNA-seq) technologies enabled unprecedented insights into the PDAC immune TME landscapes (29–31). scRNA-seq profiling of primary and metastatic PDAC specimens revealed that $CD8^+$ T cells, when present, showed expression of exhaustion markers, which were more pronounced in late-stage disease, suggesting a progressive immune dysfunction (30). Interestingly, the presence of exhausted $CD8^+$ T cells, regulatory T cells (Treg), and NK cells was associated with expression of the immune checkpoint TIGIT, opening potential new avenues for novel and more effective immunotherapies for such patients (refs. 30–32; Fig. 1C). Indeed, targeting the CD155/TIGIT axis by combinatorial immunotherapy (TIGIT + PD-1 blockade with CD40 agonism) elicited potent antitumor immune responses in preclinical *in vivo* models (32).

Taken together, these data indicate that mainly immunosuppressive cell types infiltrate PDAC and that T cells when present lack markers of activation, proliferation, and cytotoxicity. Below we will discuss the major cell types involved in the various immunosuppressive phenotypes of PDAC.

IMMUNE CELL–MEDIATED IMMUNOSUPPRESSION

The Innate Immune System: Immunosuppressive Myeloid Cell Types

An inflammatory reaction dominated by myeloid cells, such as TAMs and MDSCs, is common in patients with PDAC. TAMs originate from infiltrating monocytes or tissue-resident macrophages (33). These cells show high plasticity and exist in a spectrum of polarization states. Based on *in vitro* assays, TAMs have been classified into two extreme polarization states. M1-like TAMs are considered to have antitumor activity; are antigen-presenting cells; and express IL12, TNF, and inducible nitric oxide synthase. In contrast, M2-like cells show protumorigenic and immunosuppressive properties. They secrete Arginase 1 (ARG1), which processes and depletes L-arginine, important for T-cell function (34). In addition, they express less antigen-presenting MHC II and secrete IL10 and TGFβ, both shown to be highly immunosuppressive (35–37). TAM phenotypes are more fluid within the TME *in vivo*, where these cells are exposed to a complex milieu of polarization signals. Some

Figure 1. Heterogeneity of TME composition and organization, and context-specific modes of immunosuppression across patients with PDAC. **A–C,** PDAC patients show profound differences in the cellular composition and organization of the TME, which results in distinct TME subtypes (**A**), cell-to-cell interaction and communication (**B**), and function (**C**). As a consequence, different modes of immunosuppression exist in distinct TME subtypes of PDAC (**C**). Functions of the cell-to-cell interactions highlighted in yellow in **B** are depicted in **C**. Left, $CSF1R^+$ tumor-associated macrophages (TAM) are recruited to the tumor via cancer cell–derived secretion of CSF1, thereby promoting an immunosuppressive TME and inhibiting T-cell function. Middle, CXCL12 released by cancer-associated fibroblasts (CAF) prevents T-cell tumor infiltration. Right, neoantigens released by dying cancer cells in the TME are captured by dendritic cells for processing. After homing to the lymphoid organs, dendritic cells present the neoantigens to T cells, inducing their priming, activation, and clonal expansion. Activated T cells migrate into the TME, where they can exert anticancer immune responses through secretion of molecules such GZMB and IFNγ. However, immunosuppressive mechanisms controlled by the cancer cells, such as activation of immune checkpoints (e.g., PD-L1 or TIGIT), render them dysfunctional, thereby allowing tumor cells to evade immune destruction. **D,** PDAC patients with a high content of myeloid cells in the TME have a worse disease prognosis, whereas patients with tumors with high lymphocytes have a better overall survival. ECM, extracellular matrix; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell.

PDAC tumors secrete high amounts of colony-stimulating factor 1 (CSF1) and CC-chemokine ligand 2 (CCL2) to promote the recruitment and polarization of macrophages (Fig. 1C). Indeed, CSF1R-positive TAMs have been shown to infiltrate PDAC (38), and secretion of CCL2 from the tumor is critical for the recruitment of CCR2$^+$ monocytes from the bone marrow to the circulation and finally to the tumor, where they then differentiate into TAMs (39). Accordingly, PDAC patients with high circulating monocytes show a worse overall survival (40).

The second major immunosuppressive cell type in PDAC is represented by MDSCs. These immature myeloid cells are present in both the blood and the tumor and suppress T-cell proliferation and activation. They secrete high levels of ARG1 and reactive oxygen species and produce nitric oxide, and high abundance of MDSCs in circulation or bone marrow has been linked to tumor progression (41). MDSCs and TAMs usually dominate the PDAC TME, already in preneoplastic lesions. Notably, MDSCs are typically recruited to the tumors by a set of tumor-secreted factors, including CXCR2 ligands and GM-CSF (42), potentially opening new therapeutic options.

The Adaptive Immune System: Immunosuppressive T Cells

Disease outcomes can vary depending on the differentiation and activation status of T cells, which can be either tumor restraining via antigen-restricted immune responses or tumor-promoting via induction of immune suppression. A lack of CD8$^+$ T cells and low levels of neoantigens in combination with Th2 T cells and CD4$^+$ Tregs are associated with a tumor-permissive anergy (43–46). IL4 and IL13, Th2 cytokines, have been shown to suppress immune responses to tumor cells and drive the proliferation of *KRAS*-mutant cells (47). In the TME of PDAC, Tregs are the most abundant CD4$^+$ T-cell population. They infiltrate PDAC early, since in a KRAS-driven mouse model of PDAC, they have been shown to localize in the proximity of precursor lesions during the initial stages of tumorigenesis (48). Accordingly, Tregs have also been observed in human preneoplastic lesions, and their abundance increases with tumor progression. Moreover, high infiltration of this cell type has been associated with poor prognosis in patients with PDAC (46). In contrast to other immunosuppressive TME cells, the role of Tregs is controversial in PDAC. Historically, Tregs are considered a tumor-promoting cell type, and various mechanisms have been proposed that lead to CD8$^+$ T-cell suppression, including competition for access to antigen-presenting DCs (49). In an orthotopic transplantation model of PDAC, Tregs have been shown to promote PDAC development by engaging with tumor-associated DCs and reducing the expression of costimulatory ligands necessary for CD8$^+$ T-cell activation (50). In line, the ablation of Tregs in this model led to an increase in tumor-infiltrating CD8$^+$ T cells and blocked tumor growth (50). More recently, the immunosuppressive role of Tregs has been challenged. A publication revealed that Treg depletion in a genetically engineered mouse model (GEMM) of PDAC does not prevent immunosuppression but accelerates tumor progression (51). This study suggests that by depleting Tregs, αSMA$^+$ CAFs, which are one of the key TGFβ-producing sources in PDAC, undergo reprogramming and increase the secretion of chemoattractants for suppressive myeloid cells, which promote tumor progression (51). This suggests that Treg reprogramming, rather than depletion, could be beneficial for PDAC treatment.

STROMAL COMPONENTS OF IMMUNOSUPPRESSION

CAFs

CAFs are constituents of the desmoplastic reaction involved in the synthesis of ECM and vessel remodeling. They are most abundantly present in tumors of the classical subtype, in which they can constitute up to 80% of all cells. CAFs are a very heterogeneous population, in terms of both cell of origin and function. Even though most CAFs have been shown to originate from the activation and expansion of fibroblasts when found in proximity to tumor cells, some studies reported their origin from adipocytes, pericytes, bone marrow–derived mesenchymal stem cells, and ECs (52). In the pancreas, CAFs are thought to originate from pancreatic stellate cells, which are quiescent resident mesenchymal cells, that upon activation express αSMA and secrete tumor-promoting factors (53). CAFs dynamically evolve with tumors, and their secretome can positively and negatively modulate both cancer progression and tumor immunity via release of growth factors, cytokines, and chemokines.

In the context of PDAC, three different CAF subpopulations have been identified by scRNA-seq analysis of mouse and human tumors (54). Two of these were observed in several GEMMs of PDAC, namely, αSMA-expressing, ECM-producing myofibroblastic CAFs (myoCAF) and inflammatory CAFs (iCAF), expressing cytokines and chemokines such as IL6 (54). Another feature distinguishing these two populations is their different location within the tumor, with myoCAFs being closer to tumor cells and iCAFs more distant, potentially indicating different modes of CAF–tumor interaction (54). A smaller population of CAFs originating from mesothelial cells, with antigen-presenting function (apCAF) and MHC class II and CD74 expression, but lacking classic costimulatory molecules has also been identified in GEMMs of PDAC (52, 54, 55).

The functional role of CAFs in restraining and promoting PDAC has been studied intensively in the last years. CD105 expression is a marker denoting two functionally distinct pancreatic fibroblast lineages, with the CD105$^+$ population being permissive for tumor growth and CD105$^-$ CAFs being tumor suppressive (56). PDAC CAFs can promote tumor progression not only via paracrine or direct interactions with cancer cells but also indirectly by mediating immunosuppression. CAFs have been shown to impair antitumor T-cell responses via CXCL12 secretion, which is likely to promote the spatial exclusion of T cells, as pharmacologic inhibition of the interaction of CXCL12 with its receptor CXCR4 promoted T-cell accumulation in tumor centers and fostered efficacy of ICB (ref. 57; Fig. 1C); another mechanism includes ECM deposition, which has been shown to prevent T-cell proximity to tumor cells (see the following "ECM" section; ref. 58). Further, myoCAFs produce high amounts of TGFβ, which blocks T-cell function (36, 37). Finally, apCAFs have been shown to be able to present antigens to T cells *in vitro*

in the absence of the expression of costimulatory molecules, preventing T-cell engagement with professional antigen-presenting cells (54). *In vivo*, apCAFs have been reported to directly ligate and induce naïve $CD4^+$ T cells into Tregs in an antigen-specific fashion, thereby exerting direct immunomodulatory functions (55).

CAF-mediated immunosuppression goes beyond T cells. iCAFs are one of the most prominent sources of IL6 in PDAC (59), which promotes the differentiation of suppressive MDSCs (60). Given the double role of CAFs, their functional investigation is of fundamental importance to fully understand how to efficiently reprogram and target them.

ECM

The ECM of PDAC shows profound variation across tumors and differs fundamentally from that of the normal pancreas. It is composed of fibrillar collagens, fibronectin, elastin, laminins, and hyaluronan, produced by PDAC cells as well as CAFs, and constitutes in some tumors up to 90% of the tumor mass (61–63). ECM composition and organization strongly affect mechanical, biophysical, and chemical properties of the tumor, such as stiffness and density, as well as intratumoral signaling and communication (63–65). Further, ECM composition and organization strongly affect diffusion of nutrients and metabolites and are drivers of hypoxia and metabolic stress. All of these components and features of the ECM have been shown to mediate or attenuate immunosuppression. Indeed, the dense ECM can constitute a physical barrier that traps and prevents tumor infiltration by lymphocytes (63–65); hypoxia can mediate immunosuppression by upregulation of immunomodulatory factors like IL10, TGFβ, or VEGFA and induction of angiogenesis, all of which impede T-cell function and extravasation. In addition, VEGFA can modulate the expression of inhibitory checkpoints on $CD8^+$ T cells in tumors (65, 66). Metabolic competition between tumor and immune cells results in the deregulation of energy metabolism (67). Lactate accumulation and acidosis, lack of carbon and amino acid sources by poor nutrient availability, and the accumulation of lipids have been shown to block T-cell activation, effector function, and antitumor immunity (68–72). In PDAC, however, the role of the ECM in mediating immunosuppression is controversial, and conflicting results have been published (52, 57, 73, 74). This is most likely due to context-dependent functions of the ECM and its individual components in PDAC subtypes, as well as technical and methodologic limitations of the models used to study the contribution of the ECM to immunosuppression. Therefore, investigation of individual ECM components produced by distinct cell types using adequate model systems is needed to uncover the distinct context-specific mechanisms of immunosuppression and identify targets for therapeutic intervention (75, 76).

The Vascular System and Nerves

Tumors need to promote the formation of new vessels to ensure that the complex aggregate of tumor cells, stroma, and immune cells receives both oxygen and nutrient supplies (77). This usually abnormal vascular network is further compromised in the desmoplastic stroma–rich PDAC subtype, which causes high interstitial pressure (62). The resulting reduced perfusion promotes a hypoxic environment within the tumor and the TME, limiting the infiltration of immune cells and promoting tumor cell proliferation (78, 79). Even though the TME of PDAC has been proposed to be hypovascularized (80), the density of its microvessels can vary substantially across tumors and their abundance, relative to the stromal presence, is associated with poor survival (81). Early reports showed that human PDAC cell lines and resected tumor tissues produce high levels of VEGFA (82). VEGFA has been shown to promote EC proliferation (83) and to enhance the expression of PD-1 and other inhibitory checkpoints involved in $CD8^+$ T-cell exhaustion (65, 66). Further, *in vitro* studies determined that VEGFA expression is regulated by activated HIF1α and STAT3 in hypoxic conditions (84, 85). In addition, blood vessels are crucial in controlling the infiltration of immune cells into the tumor by expression of distinct adhesion molecules, such as ICAM1 and VCAM1, vascular permeability, and pericyte coverage. Accordingly, ECs of the tumor vasculature are able to block antitumor immunity via recruitment, adhesion, function, and killing of effector T cells (86). In addition to angiogenesis, necrosis, which is abundant in PDAC, induces immunosuppression by necroptosis-dependent CXCL1 and Mincle signaling, which has been shown to promote macrophage-mediated immunosuppression (87).

Neural invasion is one of the hallmarks of PDAC and is correlated with poor clinical outcomes (88, 89). Nerves support PDAC cell survival, migration, and angiogenic signaling by releasing neurotrophic and growth factors, as well as neurotransmitters, such as nerve growth factor, glial cell line–derived neurotrophic factor, stromal cell derived factor-1 (SDF1), adrenaline, noradrenaline, and acetylcholine (Ach). They are also part of immunomodulatory parasympathetic and sympathetic neural circuits and affect the function of immune cells by promoting protumorigenic inflammation via MDSCs and NK cells, as well as modifying the expression of inhibitory immune checkpoints, such as PD-1 and PD-L1 (88, 89). In line, use of β-blockers that target the sympathetic nervous system increased the survival of patients with PDAC (90). Additional studies and mechanistic insights are, however, needed to uncover the functional role of nerve–immune interactions and to identify targets of their cross-talk.

WHAT ARE THE DRIVERS OF THE DIVERSE TME SUBTYPES AND MODES OF IMMUNOSUPPRESSION?

Even though it has been recognized that profound differences in TME structure and organization exist and that inflammation, obesity, and smoking are important risk factors for PDAC development (91), not much is known about the cancer cell– and host-derived instructors of immunosuppression and the pro- and antitumorigenic cross-talk between PDAC cells and the surrounding stromal and immune cell populations (92–94). Interestingly, only a few studies so far have focused on how tumor cells of different subtypes, and specific features of the host, instruct their corresponding TME and drive immunosuppression. In the following paragraphs, we will describe how the context-specific composition and function of the immunosuppressive TME is controlled (i) by tumor cell–intrinsic cues, such as oncogenic KRAS signaling, as well as

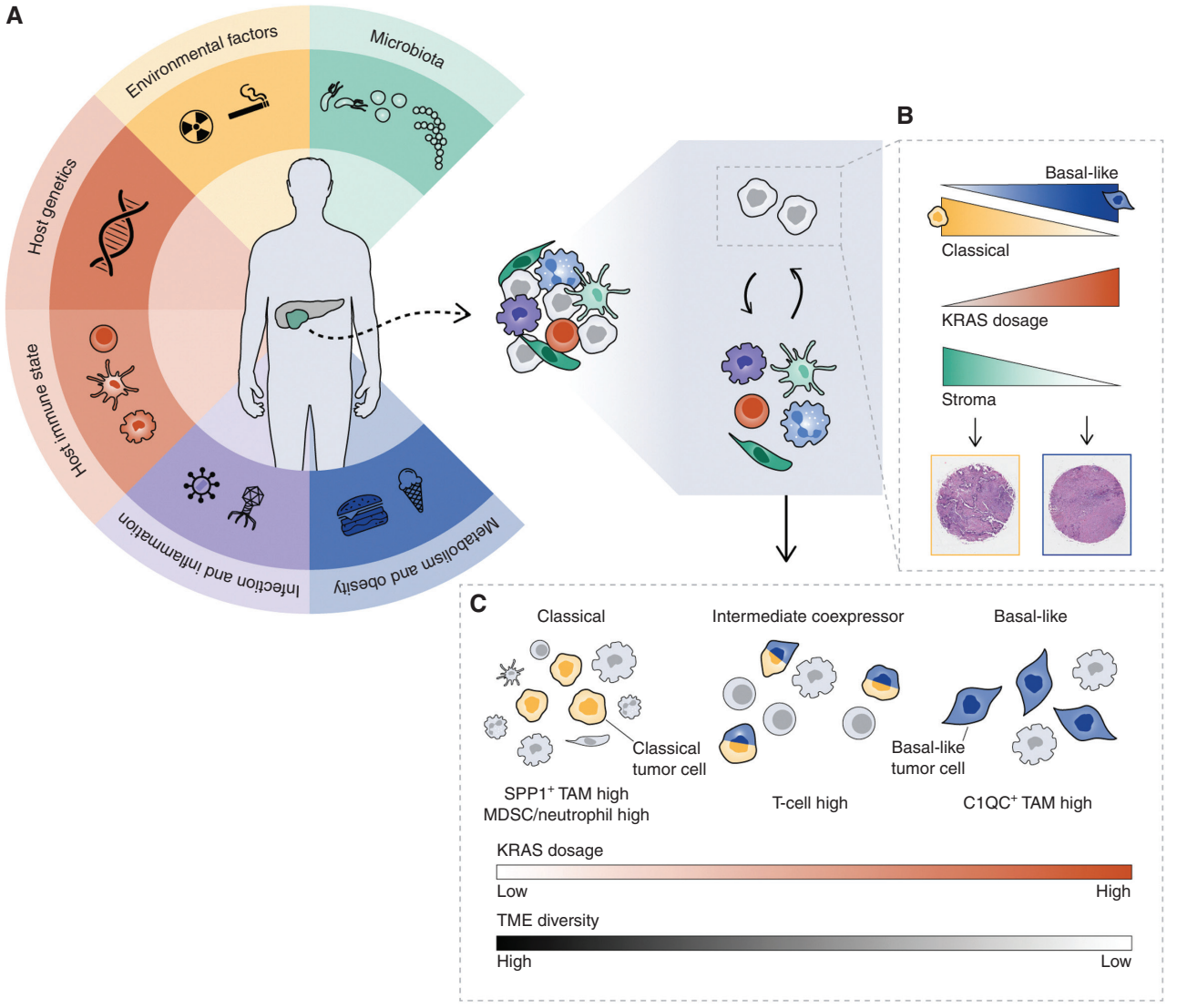


Figure 2. The context-specific composition and function of the immunosuppressive TME are controlled by tumor cell–intrinsic cues, as well as non–tumor cell–autonomous factors of the host. **A,** Context-dependent features of the host, such as genetic variation, acute and chronic infection, inflammation and injury, nutrition and metabolism, diabetes and obesity, environmental toxins, and composition of the microbiome, virome, and fungome, affect immune escape and immunosuppression. These factors constitute fundamental determinants of PDAC heterogeneity. **B,** Cancer cells of different PDAC subtypes and associated tumor cell–intrinsic signaling programs instruct their corresponding TME and drive immunosuppression. Classical and mesenchymal basal-like PDAC differ in cell morphology, gene expression programs, *KRAS* dosage, and stromal content, resulting in tumor entities with unique features that drive differences in the composition and function of their immunosuppressive TME (11, 95, 96). **C,** Tumor cell states, with distinct levels of *Kras* dosage, show differences in infiltrating immune cells and their TME. Classical tumors display high TME diversity and infiltration of SPP1$^+$ TAMs, intermediate coexpressor tumors show high T-cell infiltration, and basal-like tumors are characterized by infiltration of C1QC$^+$ TAMs (152).

(ii) non–tumor cell–autonomous factors, including the tumor micro- and macroenvironment, genetic variation of the host, as well as environmental chemicals and toxins (Fig. 2A–C).

CANCER CELL–DRIVEN IMMUNOSUPPRESSION

During PDAC evolution, oncogenic *KRAS* copy-number variation, expression, and signaling have been shown to determine important phenotypes, such as tumor cell differentiation, plasticity, histopathology, and clinical outcomes (11, 95). Indeed, the most undifferentiated subtype of the disease shows the highest *KRAS*^{G12D} gene dosage and gene expression (*KRAS*^{iGD}), reflecting its increased aggressiveness, metastatic potential, and poor prognosis (refs. 11, 95, 96; Fig. 2B and C).

Many studies have demonstrated that the effect of oncogenic KRAS goes well beyond a sustained proliferation signal in cancer. Indeed, altered signaling pathways in tumor cells play an important role in regulating the TME, with KRAS being a central hub of immunosuppression (97–99). For example, mutant KRAS is involved in the inhibition of innate and adaptive antitumor immunity via autophagocytosis-mediated downregulation of MHC I (100, 101) and modulation of PD-L1 and CD47 expression (102, 103). Oncogenic KRAS signaling has also

been shown to stimulate the uptake of extracellular proteins via macropinocytosis, which supplies cancer cells with amino acids, thereby depleting nutrients in the TME (104). Thus, KRAS induces a deregulation of TME energy metabolism and potentially drives the metabolic competition between tumor and immune cells (see also the "ECM" section above).

MYC is another important oncogene mediating cancer cell–driven immunosuppression. In mouse models, acute *Myc* activation triggers TME and immune changes reminiscent of human PDAC (105), and concomitant MYC and KRAS expression leads to advanced PDAC and suppression of type I interferon regulators IRF5, IRF7, STAT1, and STAT2, resulting in the reduced infiltration of NK and B cells and immune evasion (99). Mechanistically, MYC suppresses a TANK-binding kinase (TBK1)–dependent pathway that links double-stranded RNA metabolism with antitumor immunity (106).

By means of a focused *in vivo* CRISPR screen targeting epigenetic and RNA-binding factors in PDAC cells, lysine demethylase 3A (KDM3A) was identified as a regulator of PDAC's immune TME. Tumors lacking KDM3A showed an increase in tumor-infiltrating T cells and DCs and a decrease in myeloid cells. Mechanistically, KDM3A regulates EGFR in cancer cells through Krueppel-like factor 5 (KLF5) and SMAD family member 4 (SMAD4), and EGFR inhibition promoted a T cell–rich environment *in vivo*, highlighting the potential of EGFR targeting as an immunotherapy-sensitizing strategy (107).

So far, most studies considered PDAC as a unique tumor entity, failing to consider that this tumor is highly heterogeneous, characterized, for example, by different levels of oncogenic KRAS, TME landscapes, and molecular subtypes. A recent study integrating scRNA-seq with analyses of The Cancer Genome Atlas datasets pointed toward a higher immune infiltration in KRAS-independent/low PDAC in comparison with the KRAS-dependent/high counterpart (98). Moreover, orthotopic transplantation experiments performed with tumor cells of both subtypes highlighted a substantial difference in myeloid infiltration between tumors. Non-$KRAS^{iGD}$ tumors showed higher levels of MDSCs/neutrophils, whereas $KRAS^{iGD}$ tumors were characterized by high abundant TAMs (96). The comparison of a library of KPC-derived PDAC cell clones revealed distinct patterns of immune cell infiltration and T cell–high (inflamed) versus T cell–low (noninflamed) TMEs upon orthotopic implantation into immunocompetent mice. Further analysis uncovered a central role for tumor cell–secreted CXCL1, which is regulated via MYC in concert with epigenetic determinants to promote a T cell–depleted environment. In line, the deletion of CXCL1 induced T-cell infiltration and sensitized the tumors toward combinatorial immunotherapy (108).

These studies show the necessity to consider PDAC more holistically in all its heterogeneous phenotypes. It is evident that oncogenic KRAS and MYC both mediate tumor cell–intrinsic effects and modulate important cross-talk with the TME, specifically by promoting immune evasion and tumor progression. Additional studies are essential to investigate the context-specific role of oncogenic *KRAS* and MYC dosage systematically and functionally in modulating the composition of the TME and driving immunosuppression.

Moreover, understanding the differences between primary and metastatic PDAC TMEs and how cancer cells escape immune attack in circulation is an important future challenge (109–111). This is especially relevant given the fact that metastases to the liver and to the lung—two of the most common metastatic sites for PDAC—are correlated with different clinical outcomes and treatment responses (112), suggesting the existence of distinct immunosuppressive niches in different tissue types and metastasis sites. Platelets and granulocytes, recruited via CXCL5 and CXCL7 signaling, might play a role in immune escape in the blood stream, as shown in a model of colon cancer metastasis (113). However, systemic dysfunction of the immune system has been described as well in a variety of cancer types, including PDAC, indicating a complex cross-talk between systemic and tissue-specific cues in mediating immunosuppression during the metastatic process (109–111, 114, 115).

NON–TUMOR CELL–AUTONOMOUS MECHANISMS OF IMMUNOSUPPRESSION

In addition to cancer cell–intrinsic programs, context-dependent features of the host influence immune responses and TME features, adding another layer of complexity to the diverse immunosuppressive landscapes of the PDAC TME. Genetic variation, acute and chronic infection, inflammation and injury, metabolism, diabetes and obesity, physical activity, environmental toxins, and the composition of the microbiome, virome, and fungome all have the potential to drive or alter immune escape and immunosuppression (Fig. 2A). These non–tumor cell–autonomous factors thus constitute a second layer of context to the diversity of PDAC's immunosuppressive TME landscapes. However, in PDAC, the mechanistic investigation of host-derived genetic and micro- and macroenvironmental factors that influence the organization of the peripheral immune system and drive immunosuppression is clearly an underinvestigated field. In the following paragraphs, we summarize our current knowledge of host and environmental factors driving immunosuppression in PDAC and pinpoint the many translationally relevant open questions and important future directions of research.

Genetic Variation of the Host

Genetic and epigenetic factors have been shown to strongly affect variation of immune cell function and immune responses in humans (116). The immune system itself displays a massive interindividual diversity; immunity is controlled by highly polymorphic genes as well as environmental cues. Genetic profiling revealed that several thousand genetic loci with weak individual effects drive up to 50% of the observed immune variation (116). Importantly, the expression of cytokines, which are among the most important drivers of immunity, displays an extraordinarily high degree of hereditability (117). In addition to genetic variation, gender and age, diet and environmental factors, and the microbiome affect the residual variation in immune function. Thus, genetic variation is an important driver of immune variation and the different types of immune responses, such as Th1, Th2, or Th17, as well as type I interferon or inflammasome activation (116).

In PDAC so far, mainly association studies have been performed that revealed that genetic and epigenetic variation in cytokines and their receptors, such as IL6, IL8, IL10, TNF,

and TGFβ, risk factors for inflammatory bowel disease, such as NOD1, or genes regulating Th1/Th2 immune responses are linked with increased cancer risk or altered survival of patients with PDAC (118–120). However, the impact of these variations on antitumor immunity and the immunosuppressive TME of PDAC subtypes remains largely elusive.

One of the few examples of investigating immune-related functions of genes identified in genome-wide association studies with increased PDAC risk is *NR5A2*. An elegant study uncovered a gene dosage–dependent function of NR5A2 in suppressing inflammatory programs in the pancreas, which have been shown to drive PDAC progression (121). Furthermore, it has been shown that targeting the proinflammatory tumor-promoting cytokine IL6 with neutralizing antibodies in mice sensitizes orthotopic PDAC to anti–PD-L1 ICB and increases intratumoral effector T-cell abundance (122). Importantly, immune variation can also limit immunotherapeutic approaches by triggering side effects. ICB inhibitors induce adverse immune effects, such as autoimmunity in up to 50% of the treated patients (123). Interestingly, persons with allergies are protected against PDAC, supporting the notion that individuals with an overactive immune system display increased antitumor immunity (91). A better understanding of host genetic variants that drive immune variability and shape the immunosuppressive TME will help to stratify patients and develop novel precision medicine strategies with reduced side effects.

Infection and Inflammation

The link between infection, inflammation, and cancer is well established, and we refer to other reviews that discuss the consequences of unresolved infections and chronic inflammation on immunosuppression and antitumor immunity in the TME (93, 124). In PDAC, chronic bacterial (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, CagA–H. pylori*) and viral (hepatitis B and C virus) infections, as well as chronic pancreatitis, have been linked to greater cancer risk and tumor progression (91, 125). In addition, there is evidence that acute inflammatory injuries of the pancreas also drive tumor progression (126, 127). Inflammation has been shown to create a protumorigenic and immunosuppressive TME via the recruitment of immunosuppressive immune cells, such as TAMs, MDSCs, and Tregs (127, 128). Inflammation induces the secretion of inflammatory mediators, such as growth factors (i.e., TGFβ) and cytokines (i.e., IL1β and IL6), which promote protumorigenic inflammation and shape the TME toward a tumor-permissive state that suppresses immune responses via the deactivation of T cells in the TME (127, 129). As an example, TGFβ, which has a major role in fibrotic reactions in chronic pancreatitis, is also a major local immunosuppressor (35, 128, 129). We believe that understanding the so far unknown context-dependent inflammatory signals that recruit immunosuppressive immune cells to the tumor site and the functional program of these cells that mediate immunosuppression may help to target the distinct modes of immunosuppression of PDAC subtypes more efficiently.

Obesity and Metabolic Diseases

Cancer risk and the likelihood of death from PDAC are increased in metabolic disorders, such as obesity and type 1 or type 2 diabetes, whereas it is decreased by aerobic exercise (91, 130). Obesity and diabetes mellitus, which are diagnosed in up to 60% of patients with PDAC, have been shown to induce metabolically driven inflammatory (metaflammatory) signals and chronic subclinical inflammation (131). Accordingly, obesity induces steatosis, inflammation, and fibrosis in the normal pancreas (132). In GEMMs of human PDAC, obesity drives immunosuppression and tumor progression via hypertrophic adipocytes that accumulate in the TME, which can secrete proinflammatory cytokines, lipids, and adipokines, such as IL1β, TNFα, and lipocalin-2 (LCN2; refs. 131–133). IL1β released from adipocytes, for example, activates stellate cells and increases desmoplasia and the infiltration of immunosuppressive neutrophils in PDAC models, which can be blocked by IL1β inhibition (132). Obesity also increases the secretion of the adipokine LCN2. LCN2 has been shown to activate stellate cells and remodel the stroma toward immunosuppression by recruiting TAMs into the tumors (133). In contrast, aerobic exercise reduces PDAC growth by reprogramming the immunosuppressive TME via IL15-mediated mobilization and accumulation of IL15Rα$^+$ CD8 T cells and sensitizes PDAC to ICB (130).

Environmental Factors, Nutrition, and the Microbiome

Exposure to environmental toxins, such as alcohol and smoke, constitutes risk factors for PDAC development (91). Smoke not only increases the mutational load of the tumors (134, 135) but also contributes to immunosuppression in a context-dependent manner in different cancer types, including PDAC (136, 137). In a KRAS-driven mouse model of PDAC, it has been shown that smoke exposure leads to the activation of pancreatic stellate cells and the induction of TAM differentiation, thereby creating an immunosuppressive microenvironment (136). However, the underlying molecular mechanisms are entirely unclear so far. Response rates to ICB correlate with the mutation rate of the tumors. Whether smoke-triggered PDAC is more sensitive to ICB remains to be determined.

Nutrition influences inflammatory and immune responses substantially, and cancers critically depend on nutrients for their growth and viability. Quantitative or qualitative dietary interventions can alter nutrient availability and immunity in the TME, which represents an attractive possibility to increase the efficacy and reduce the side effects of combination (immuno)therapies. Quantitative and qualitative variations of nutrient uptake, such as overfeeding and fasting, have been shown to display strong immunomodulatory effects in the TME (129). Hypercaloric, high-fat, and Western-style diets induce chronic subclinical inflammation in normal tissue types and an immunosuppressive TME in various cancer types (129). For example, activation of the lipid nuclear receptor peroxisome proliferator-activated receptor-delta (PPARδ) by a high-fat diet leads to TME remodeling and pancreatic intraepithelial neoplasia progression to PDAC in a KRAS^{G12D}-driven mouse model. Mechanistically, PPARδ activation in epithelial cells induced secretion of CCL2, thereby promoting an immunosuppressive TME via the recruitment of TAMs and MDSCs (138). Moreover, it has

been shown recently that the mitochondrial glutamic-oxaloacetic transaminase GOT2 directly binds to fatty acid ligands to induce PPARδ, resulting in the infiltration of ARG1$^+$ TAMs and lack of T cells (139).

Altering the metabolic environment in the TME can change not only the metabolic activity of cancer cells but also immunometabolism. For example, dietary arginine supplementation has been shown to induce global metabolic changes in T cells, such as a shift from glycolysis to oxidative phosphorylation, which increases T-cell activation and T cell–mediated antitumor immune responses in a mouse model of melanoma (34). Fasting has anti-inflammatory effects and increases the abundance of tumor-infiltrating lymphocytes and reduces PD-L1 expression in different tumor models (129, 140, 141). However, the effects of individual nutrients on immunosuppression and T-cell function in the TME have not been investigated in detail and are completely elusive in PDAC. One exception are vitamins with anticancer properties, such as vitamin D, which has been shown to decrease inflammation and fibrosis in PDAC via the transcriptional reprogramming and silencing of CAFs (142).

Recent reports suggest a key role of the microbiome in PDAC initiation, maintenance, and antitumor immunity (143). Distinct patterns of the intestinal microbiome can drive cancer formation as well as treatment response and resistance both systemically and locally, for example, by stimulating immune cells to secrete inflammatory cytokines, thereby inducing inflammation and immunosuppression (129, 143–145). In addition, metabolites of the diet, generated by the intestinal microbiota, can promote an immunosuppressive microenvironment. Tryptophan metabolites such as indoles have been shown to activate the aryl hydrocarbon receptor in myeloid cells, thereby inducing TAM polarization and immunosuppression in PDAC (146). A recent study in mice sheds light on the role of the local microbiome in shaping tumor-associated immune responses, showing that the PDAC microbiome promotes cancer development and progression by both adaptive and innate immune suppression. Mechanistically, PDAC-associated dysbiosis drives immunosuppressive CD206$^+$ M2-like TAM polarization via Toll-like receptor 2 and 5 ligation, thereby suppressing T-cell immunity (147). Further, it has been shown that the intratumoral microbial diversity is associated with better outcomes in patients with PDAC and that a specific microbiome signature (*Pseudoxanthomonas–Streptomyces–Saccharopolyspora–Bacillus clausii*) is linked to CD8 T-cell infiltration and activation, as well as host antitumor immune responses, which could be altered by fecal microbiota transplantation experiments in mice (148).

In addition to bacteria, fungi (*Malassezia spp*.) have also been shown to promote inflammation and PDAC progression by activating the C3 complement cascade via ligation of mannose-binding lectin (MBL; ref. 149). The role of the virome and phages in modulating immunosuppression in PDAC has not been explored yet.

Importantly, the tumor microbiome shows significant differences among PDAC subtypes and is associated with distinct context-dependent inflammatory signatures; however, the underlying mechanisms that drive these differences and their functional consequences are unknown (150). Therefore, the role of the microbiome in PDAC subtype specification and oncogenic signaling output is relevant to be further addressed experimentally. In addition, it remains to be determined how the context-dependent composition of the microbiome in turn modulates immune responses and mediates immunosuppression in PDAC subtypes. This knowledge might further contribute to our understanding of the critical role of microbes in shaping the immunosuppressive TME and represents novel possibilities to elicit immune responses by modulating the microbiota.

IMMUNOSUPPRESSION IN CONTEXT

A recent elegant study discovered that tumor cell–intrinsic epigenetic reprogramming and transcription factor networks contribute to tumor immune plasticity and PDAC subtype differentiation. The basal-like mesenchymal PDAC subtype is sustained by a BRD4-mediated cJUN expression program via CCL2 secretion, which leads to the recruitment of inflammatory TAMs that produce TNFα, thereby maintaining the mesenchymal phenotype. This finding opens avenues for the use of BRD4 inhibitors (e.g., JQ1) to induce redifferentiation with the aim to switch mesenchymal PDAC to a classical, therapy-sensitive phenotype, characterized by a more favorable prognosis (ref. 151; Fig. 3A). Single-cell analyses are in this context of fundamental importance to match transcriptional profiles and TME phenotypes. For example, a recent study investigating scRNA-seq of matched metastatic PDAC and organoid cultures identified two transcriptional signatures, namely, single-cell basal (scBasal) and single-cell classical (scClassical), mostly matching with previously established transcriptional subtypes (ref. 14; Box 1). Interestingly, and in line with other publications investigating PDAC subtypes with single-cell technologies (29), the authors observed that these tumor cell states were not mutually exclusive, and certain samples had cells with intermediate gene expression of the defined markers. These three transcriptional programs were also associated with different TME compositions. scClassical tumors showed a high Simpson's diversity index (a measure of diversity taking into account the number of cell types present), indicating a heterogeneous TME, whereas scBasal tumors presented a more homogeneous TME. The TME of scClassical tumors showed an infiltration of SPP1$^+$ TAMs, which are characterized by the upregulation of genes involved in angiogenesis, whereas the scBasal TME lacked CD8$^+$ T cells and was dominated by C1QC$^+$ TAMs, showing preferential expression of genes involved in phagocytosis and antigen presentation. T cells were positively correlated with the intermediate state (ref. 152; Fig. 2C). Another stratification strategy making use of scRNA-seq and proteomics approaches revealed the existence of "sub-TMEs," regional and recurrent TME phenotypes associated with distinct immune and CAF composition but also with distinct prognosis and response to therapy, highlighting the high grade of intratumor heterogeneity in PDAC. Deserted sub-TMEs were immunologically cold; were characterized by thin, spindle-shaped CAFs; and were associated with poor treatment response in patients. Vice versa reactive sub-TMEs displayed an immunologically hot TME, with CAFs showing enlarged nuclei, and a good

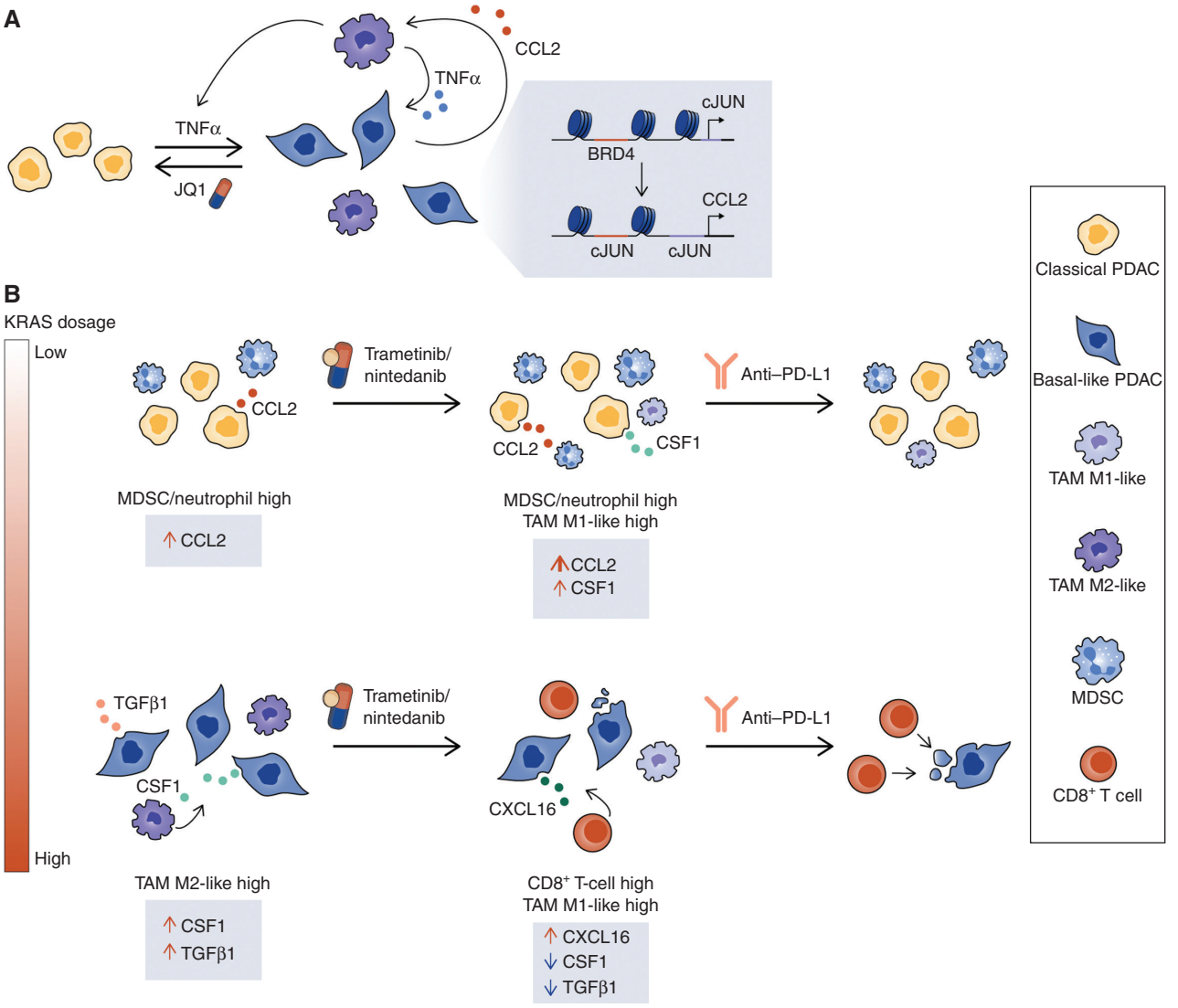


Figure 3. Therapy-induced reprogramming of PDAC subtypes and their immunosuppressive TME. **A,** Basal-like mesenchymal PDAC relies on BRD4-dependent cJUN/AP1 expression, which induces CCL2. CCL2 secretion leads to the recruitment of TNFα-secreting macrophages, which promote reprogramming of classical tumor cells into basal-like mesenchymal ones and maintenance of the mesenchymal state. The use of BRD4 inhibitors such as JQ1 suppresses the BRD4–cJUN–CCL2–TNFα axis and induces redifferentiation of the mesenchymal to the classical PDAC subtype, which is characterized by a more favorable prognosis (151). **B,** Classical and basal-like mesenchymal PDAC are driven by tumor cell–intrinsic cues (e.g., *KRAS* dosage), and their TME is characterized by distinct immune cell infiltrates. This results in a differential response to a combinatorial therapy of the MEK inhibitor trametinib and the multikinase inhibitor nintedanib (T/N). The combination promotes the context-dependent reprogramming of the tumor cell secretome, thereby inducing a subtype-specific TME remodeling. In the classical subtype, T/N induces infiltration of MDSCs/neutrophils and M1-like TAMs and does not sensitize the tumors to anti–PD-L1 ICB. In basal-like mesenchymal PDAC, T/N leads to the recruitment of M1-like TAMs and $CD8^+$ T cells, sensitizing the tumors to anti–PD-L1 ICB (96).

response to chemotherapy. Patients showing the co-occurrence of both displayed worse outcomes (153).

THERAPY-INDUCED IMMUNE MODULATION

Cancer treatments affect not only the tumor cells but also the surrounding TME, resulting in changes of the composing cell types (Fig. 3A and B). In PDAC, both chemotherapy and radiotherapy increase the abundance of TAMs in tumors, leading to an immunosuppressive and tumor-promoting environment (154, 155). Radiotherapy has also been reported to alter CAFs, leading to elevated ECM production, which promotes PDAC cell survival via integrin signaling (156). In addition, a recent study identified a neural-like progenitor program of PDAC cells, enriched especially after chemotherapy and radiotherapy, which was associated with a poor patient outcome (13). Moreover, remodeling of the TME also depends on the treatment duration. For example, long-term gemcitabine treatment has been shown to induce immunosuppression in mouse models of PDAC; over time, PDAC cells expressed higher levels of PD-L1, PD-L2, MHC I, and immunosuppressive secreted factors, including TGFβ (157).

It is important to note that some therapies can induce immunogenic cell death or an antitumorigenic TME reprogramming, thereby synergizing with the treatment effect on the tumor cell compartment. For instance, in mouse models of PDAC, the combinatorial treatment with MEK and CDK4/6 inhibitors promoted a senescence-associated secretory phenotype, vascular remodeling, enhanced drug delivery, and T-cell infiltration, thereby sensitizing the treated tumors to ICB (158). Another study showed that targeting the proline polymerase PIN1 using clinically available drugs leads to CAF remodeling, induces upregulation of PD-L1 and the gemcitabine transporter ENT1, and renders PDAC tumors eradicable in combination with gemcitabine and ICB (159). Building on both context specificity and TME remodeling concepts, it has recently been shown that combining MEK and multi–receptor tyrosine kinase inhibition, making use of trametinib and nintedanib, led to subtype-specific TME remodeling in mice. The combination of inhibitors promoted the reprogramming of the immunosuppressive secretome in mesenchymal PDAC, leading to T-cell infiltration and sensitizing this highly therapy-resistant subtype to ICB treatment (ref. 96; Fig. 3B).

THE PDAC TME AS A TARGET FOR THERAPY

Therapies targeting the immune compartment have revolutionized the treatment of several malignancies (160). However, except for the 1% of patients with PDAC harboring MSI-high tumors, this has not been the case for PDAC so far (161). Putative reasons are multiple, including the relatively low tumor mutational burden compared with malignancies that respond to immunotherapies and the presence of a highly immunosuppressive TME. Given the important role of the PDAC TME in mediating treatment response, we will focus in the following paragraphs on strategies targeting some of the most abundant and immunosuppressive cell types infiltrating this tumor type and depict selected studies that target the various features of the TME and the host (Table 1).

CAFs and the ECM

Many studies have tried to modulate the desmoplastic reaction typical of the classical subtype of PDAC. Some of them targeted the ECM by altering MMP activity, hyaluronan deposition, or sonic hedgehog signaling; however, these strategies did not show sufficient therapeutic efficacy or in some cases even shortened the survival of the patients in early clinical trials (162). Direct approaches to target CAFs have initially focused on the inhibition of fibroblast-activated protein (FAP), one of the most broadly expressed proteins in fibroblasts. In a phase II, single-arm clinical trial combining gemcitabine with the FAP inhibitor talabostat, the combination therapy showed no benefit over historical gemcitabine monotherapy cohorts of patients with metastatic PDAC (162). Given the double role of CAFs in the TME, subsequent studies focused on CAF reprogramming toward a tumor-constraining phenotype. An example is represented by a recent phase II clinical trial combining paricalcitol, a vitamin D derivative, nivolumab ICB, and/or chemotherapy (NCT02754726). Another approach, which has not been tested in the clinic yet, is the use of JAK inhibitors to promote a phenotype switch from iCAFs to myoCAFs in order to downregulate the secretion of tumor-promoting inflammatory cytokines and chemokines by iCAFs (163). In addition, several studies tested ways to block CAF-mediated immunosuppression. An example is the CXCR4 antagonist BL-8040 that disrupts CAF-mediated CXCL12–CXCR4 signaling, which is currently under investigation in a phase II clinical trial in combination with chemotherapy and/or pembrolizumab (NCT02826486). Future strategies targeting immunosuppressive CAFs should aim at reprogramming the population rather than their depletion.

Myeloid Cells

Given the high relevance of myeloid cells in immunosuppression, strategies have been developed to (i) directly deplete myeloid cells, (ii) inhibit the cytokine(s) mediating the recruitment and accumulation of myeloid cells in the TME, (iii) inactivate the tumor cell–intrinsic pathway(s) driving the release of chemoattractants, and (iv) change the polarization of myeloid cells from protumorigenic to antitumorigenic. Immunosuppressive TAMs are recruited via the CSF1/CSF1R axis to the tumor. Targeting of CSF1R has been proven to reduce tumor burden, increase T-cell infiltration (38, 164), and sensitize PDAC to anti–PD-1 and CTLA-4 ICB (165). To prevent the recruitment of TAMs, CCR2 inhibitors have been used in mice. Blocking the CCL2/CCR2 axis resulted in reduced $CCR2^+$ monocyte recruitment and reduced tumor growth, and synergism in combination with standard-of-care chemotherapy (40). Similar results were observed in a phase Ib clinical trial of PDAC patients with borderline resectable and locally advanced disease treated with the combination of FOLFIRINOX and the CCR2 inhibitor PF-04136309 (166). However, PF-04136309 did not improve therapy response when combined with gemcitabine/nab-paclitaxel in a phase Ib study of patients with metastatic disease (167). Another approach that has been tested in PDAC to target TAMs is the use of CD40 agonists. The CD40 costimulatory receptor is broadly expressed on immune cells, including monocytes and macrophages, and is important to allow antigen presentation, among other functions (168). Macrophages are antigen-presenting cells, and M2-like TAMs that are highly abundant in PDAC express low levels of MHC II, suggesting that they could be reprogrammed to increase their antigen-presenting capacity. Treatment of PDAC GEMMs with a CD40 agonist induced upregulation of MHC II in TAMs, suggesting a reprogramming toward an antitumor phenotype, and increased PDAC T-cell infiltration (169, 170). In patients, treatment with a CD40 agonist antibody in combination with gemcitabine in a phase I study led to a reduction in tumor burden (169). Moreover, in PDAC mouse models, the combination of CD40 agonist with gemcitabine/nab-paclitaxel sensitized tumors to ICB (171). Based on these results, this combined chemoimmunotherapy approach is currently being tested in the PRINCE trial in patients with metastatic disease (NCT03214250). Preliminary results of the phase Ib portion of the trial showed the tolerability of the chemoimmunotherapy combination [sotigalimab (CD40 agonist) + gemcitabine/nab-paclitaxel ± nivolumab; ref. 172]. In the randomized phase II portion of the study, only a modest increase in overall survival was observed for the nivolumab/chemotherapy arms versus control, and the sotigalimab/

Table 1. Selected clinical trials investigating novel immune, TME, and host-modulating drug combinations for PDAC treatment

Drug combinations	Chemotherapy	ICB	Mechanism of additional agent(s)	Phase	*N*	Population	Clinical trial
Ciprofloxacin + gemcitabine + nab-paclitaxel	Yes	No	Ciprofloxacin: Targeting the microbiome	Phase I	10	Metastatic PDAC	NCT04523987
BMS-813160 + nivolumab + gemcitabine + nab-paclitaxel	Yes	Anti–PD-1	BMS-813160: CCR2/CCR5 antagonist	Phase I/II	40	PDAC	NCT03496662
GEN1042 + pembrolizumab ± gemcitabine + nab-paclitaxel	Yes	Anti–PD-1	GEN1042: Bispecific agonistic antibody targeting CD40/4-1BB	Phase I/II	447	Malignant solid tumors, including PDAC	NCT04083599
NZV930 + spartalizumab ± NIR178	No	Anti–PD-1	NZV930: CD73 antagonist; NIR178: adenosine 2A receptor antagonist	Phase I	344	Malignant solid tumors, including PDAC	NCT03549000
Atorvastatin + ezetimibe + evolocumab + FOLFIRINOX	Yes	No	Atorvastatin, ezetimibe, evolocumab: Cholesterol metabolism disruption	Early phase I	12	Metastatic PDAC	NCT04862260
SX-682 + nivolumab	No	Anti–PD-1	SX-682: CXCR1/2 antagonist	Phase I	20	PDAC	NCT04477343
MEDI4736 + nab-paclitaxel + gemcitabine or MEDI4736 + AZD5069	Yes	Anti–PD-L1	AZD5069: CXCR2 antagonist	Phase I/II	23	Metastatic PDAC	NCT02583477
Different combinations of the following compounds: nab-paclitaxel ± gemcitabine ± oxaliplatin ± leucovorin ± fluorouracil ± atezolizumab ± cobimetinib ± PEGPH20 ± BL-8040 ± selicrelumab ± bevacizumab ± RO6874281 ± AB928 ± tiragolumab ± tocilizumab	Yes	Anti–PD-L1 + anti-TIGIT	Cobimetinib: MEK inhibitor; PEGPH20: ECM targeting; BL-8040: CXCR4 antagonist; selicrelumab: CD40 agonist; bevacizumab: VEGF inhibitor; RO6874281: engineered variant of IL2 (IL2v) targeted to tumor-associated fibroblasts via binding to FAP; AB928: dual adenosine receptor antagonist; tocilizumab: IL6 receptor	Phase I/II	290	PDAC	NCT03193190
Fecal microbiota transplantation	No	No	Patients undergo fecal microbiota transplantation during colonoscopy	Early phase I	10	PDAC	NCT04975217
L-glutamine + gemcitabine + nab-paclitaxel	Yes	No	L-glutamine: Metabolism	Phase I	16	Advanced PDAC	NCT04634539
Canakinumab + spartalizumab + nab-paclitaxel + gemcitabine	Yes	Anti–PD-1	Canakinumab: Anti-IL1β monoclonal antibody	Phase I	10	Metastatic PDAC	NCT04581343

(*continued*)

Table 1. Selected clinical trials investigating novel immune, TME, and host-modulating drug combinations for PDAC treatment (*Continued*)

Drug combinations	Chemotherapy	ICB	Mechanism of additional agent(s)	Phase	*N*	Population	Clinical trial
CAN04 ± gemcitabine + nab-paclitaxel	Yes	No	CAN04: IL1 receptor accessory protein (IL1RAP)	Phase I/II	140	Malignant solid tumors, including PDAC	NCT03267316
NGM707 ± pembrolizumab	No	Anti–PD-1	NGM707: ILT2/ILT4 antagonist	Phase I/II	179	Malignant solid tumors, including PDAC	NCT04913337
Regorafenib + nivolumab	No	Anti–PD-1	Regorafenib: Multi-RTK inhibitor	Phase II	175	Malignant solid tumors, including PDAC	NCT04704154
IACS-010759	No	No	IACS-010759: Oxidative phosphorylation inhibitor	Phase I	29	Malignant solid tumors, including PDAC	NCT03291938
NIS793 ± spartalizumab + gemcitabine + nab-paclitaxel	Yes	Anti–PD-1	NIS793: TGFB1 inhibitor	Phase II	161	Metastatic PDAC	NCT04390763
Personalized peptide vaccine ± imiquimod ± pembrolizumab ± sotigalimab	No	Anti–PD-1	Imiquimod: Toll-like receptor 7 agonist; sotigalimab: CD40 agonist	Phase I	150	Malignant solid tumors, including advanced PDAC	NCT02600949
Ascorbic acid + nab-paclitaxel + cisplatin + gemcitabine	Yes	No	Administration of high-dose IV vitamin C	Phase I/II	27	Advanced PDAC	NCT03410030
Paricalcitol + hydroxychloroquine + losartan	Yes	No	Paricalcitol: Vitamin D analogue; hydroxychloroquine: autophagy; losartan: angiotensin II receptor antagonist	Early phase I	20	PDAC	NCT05365893
Paricalcitol + nab-paclitaxel + cisplatin + gemcitabine	Yes	No	Paricalcitol: Vitamin D analogue	Phase II	14	Advanced PDAC	NCT03415854

Abbreviation: RTK, receptor tyrosine kinase.

chemotherapy and sotigalimab/chemotherapy/nivolumab arms did not demonstrate improvements in 1-year overall survival rates (173). Prospective studies to identify biomarkers of response will be necessary to achieve higher efficacy.

MDSCs are immunosuppressive; therefore, their targeting has been proposed as an interesting therapeutic strategy for PDAC. Most of the work published so far has focused on preventing the recruitment of this cell population to tumor sites. Targeting CXCR2, a receptor present on MDSCs and neutrophils that promotes their recruitment to the tumor, resulted in an increase in survival in PDAC GEMMs and T-cell recruitment, and combined inhibition of CXCR2 and PD-1–based ICB further extended survival (174). On the other hand, targeting tumor cell–secreted GM-CSF has also shown some promise in PDAC mouse models, as it blocks the recruitment of Gr-1$^+$ CD11b$^+$ myeloid cells to the TME and tumor growth in a CD8$^+$ T-cell–dependent manner (175). These results suggest that targeting the myeloid compartment holds promise for the treatment of PDAC.

Tregs

Different approaches have been explored to target Tregs. One of the earliest examples includes the incorporation of low-dose cyclophosphamide in different treatment regimens (176). Tregs showed higher susceptibility to its toxic effects because of their low levels of intracellular ATP and glutathione (177). In addition, CTLA-4 (178) and neuropilin-1 (50) have been investigated as targets for intratumor Tregs. Moreover, Tregs can be recruited to the tumor via CCL5. Therefore, blocking CCL5/CCR5 signaling has been tested as a therapeutic approach. This prevented Treg migration to the tumor and resulted in decreased PDAC growth in mice (179). However, given the dual role of Tregs in PDAC, as discussed in the previous paragraphs, identifying therapeutic strategies

aimed at T-cell reprogramming rather than depletion could benefit PDAC outcomes (51).

CONCLUSIONS AND FUTURE PERSPECTIVES

PDAC is a heterogeneous disease, characterized by extensive intertumoral and intratumoral diversity. However, immunosuppression is a unifying feature of PDAC across its entire spectrum of phenotypic variation, considering that even MSI-high tumors display remarkable resistance to ICB. Novel combinatorial therapies aimed at promoting T-cell priming, such as chemotherapy, chemoradiation, oncolytic virotherapy, and vaccination, have been inefficient so far. Recently, we have come to understand that the immunosuppressive TME constitutes a key barrier toward effective immunotherapy in PDAC. Therefore, it is of fundamental importance to mechanistically investigate the drivers of TME organization and immunosuppression and ways to successfully target them.

Here we have presented evidence for fundamental context-dependent differences in the composition and spatial organization of the TME in PDAC subtypes, such as the amount, composition, and localization of infiltrating immune cell (sub)types, suggesting the existence of distinct immunosuppressive niches and modes of immunosuppression. These differences not only dictate the unique biology of the tumors but also affect immunotherapeutic response and resistance and are therefore one of the likely reasons for the mixed responses toward novel immunotherapeutic approaches tested currently in early-phase clinical trials. Therefore, a deep mechanistic understanding of the biology and regulatory networks that control the multiple distinct modes of immunosuppression of PDAC subtypes has the potential to open groundbreaking new therapeutic options that might enable immune-mediated or immune-assisted PDAC eradication.

In this review, we provided evidence that the context-dependent immunosuppressive TME landscapes and antitumor immune responses are shaped by (i) the molecular features of the tumor, such as genetic and epigenetic alterations, as well as (ii) the host and (iii) its environment (Fig. 2A–C). Although progress in (immuno)phenotyping of PDAC has provided considerable knowledge about the composition and organization of the cellular components of the TME and their interactions with tumor cells (13), the mechanistic bases and the signals that control the diverse context-dependent modes of immunosuppression are still largely enigmatic. This is mainly due to (i) a lack of immunocompetent *in vivo* models for PDAC TME subtypes and functional and quantitative readouts of immunosuppression/activation, which allow us to validate basic mechanisms and candidate drivers, (ii) missing large-scale high-dimensional datasets and resources that enable robust knowledge extraction to decode the critical nodes of the tumor–immune niche cross-talk of PDAC TME subtypes, and (iii) technological limitations to study cell type–specific cell communication networks on a systems-wide level *in vivo*.

Recent advances in mouse modeling, cell type–specific proteomics, and high-throughput single-cell analysis and cell profiling technologies should reveal critical insights about the cellular and molecular complexity of PDAC subtypes and the dynamic cellular interactions that drive the distinct modes of immunosuppression, as well as how to target them therapeutically. Systematic and comprehensive large-scale approaches at different time points, for example, at diagnosis, during therapy, and upon resistance, are needed to achieve this goal. However, one critical challenge will be to functionalize such cell atlases, for example, by genetic perturbations, to take full advantage of these high-dimensional datasets. This will allow us to identify novel targets and develop combinatorial immunomodulatory therapies that target the tumor, its immunosuppressive TME, and systemic factors of the host. Here, rational multimodal approaches are clearly necessary to achieve therapeutic success. Adequate strategies and tools to neutralize with high specificity the immunosuppressive stroma and host factors, such as microbes that drive immunosuppression, are essential, especially when combined with therapies that enhance T-cell priming and prevent T-cell exhaustion, as well as therapeutics that block oncogenic signaling with high efficacy. The advent of KRAS-directed therapies as well as engineered T cells (e.g., chimeric antigen receptor T-cell or T-cell receptor T-cell therapies), oncolytic viruses, and vaccination strategies (e.g., mRNA-based) will provide novel opportunities to achieve this goal.

Authors' Disclosures

D. Saur reports grants from the German Cancer Consortium (DKTK), Deutsche Forschungsgemeinschaft, Wilhelm Sander-Stiftung, and the European Research Council during the conduct of the study. No disclosures were reported by the other authors.

Acknowledgments

This study was supported by the German Cancer Consortium (DKTK), Deutsche Forschungsgemeinschaft (DFG SA 1374/4-2; DFG SA 1374/6-1; DFG SCHO 1732/2-1; SFB 1321 Project-ID 329628492 P06; and SFB 1371 Project-ID 395357507 P12 to D. Saur), the Wilhelm Sander-Stiftung (2020.174.1 and 2017.091.2), and the European Research Council (ERC CoG No. 648521 to D. Saur).

Received August 4, 2022; revised October 17, 2022; accepted October 26, 2022; published first January 9, 2023.

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