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Samantha B. Kemp

Marina Pasca di Magliano

Howard C. Crawford
Henry Ford Health, hcrawfo1@hfhs.org

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Myeloid Cell Mediated Immune Suppression in Pancreatic Cancer

Samantha B. Kemp,[1] Marina Pasca di Magliano,[2,3,4] and Howard C. Crawford[5]

[1]Department of Molecular and Cellular Pathology, [2]Department of Surgery, [3]Department of Cell and Developmental Biology, and [4]Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan; and [5]Henry Ford Pancreatic Cancer Center, Henry Ford Health System, Detroit, Michigan

SUMMARY

The immunosuppressive tumor microenvironment in pancreatic cancer is comprised in part by various myeloid cells, including tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). We discuss the role of TAMs and MDSCs in promoting immune suppression and highlight current myeloid targeted therapies.

Pancreatic ductal adenocarcinoma (PDA), the most common pancreatic cancer, is a nearly universally lethal malignancy. PDA is characterized by extensive infiltration of immunosuppressive myeloid cells, including tumor-associated macrophages and myeloid-derived suppressor cells. Myeloid cells in the tumor microenvironment inhibit cytotoxic T-cell responses promoting carcinogenesis. Immune checkpoint therapy has not been effective in PDA, most likely because of this robust immune suppression, making it critical to elucidate mechanisms behind this phenomenon. Here, we review myeloid cell infiltration and cellular crosstalk in PDA progression and highlight current therapeutic approaches to target myeloid cell-driven immune suppression.

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal human malignancies, with a 5-year survival rate of only 10%.[1] PDA is projected to become the second leading cause of cancer-related deaths by 2030.[2] This poor prognosis is due in part to most patients presenting with metastatic disease and overwhelming resistance to chemotherapy and radiotherapy approaches. The only potential cure for PDA is surgical resection, for which only 20% of patients are eligible, and ultimately 80% of these patients will relapse with local recurrence or metastatic disease.[3] Current frontline therapies are the chemotherapy regimens FOLFIRINOX or gemcitabine/nab-paclitaxel, which modestly extend survival.[4–6] The main genetic drivers of PDA are mutations in the *KRAS* oncogene,[7,8] along with loss of functional tumor suppressors (*TP53*, *SMAD4*, INK4*A*).[9,10] Both acinar cells and ductal cells within the healthy pancreas can give rise to PDA, although acinar cells appear to have a higher propensity for transformation.[11] Acinar cells go through a plastic transdifferentiation process called acinar to ductal metaplasia (ADM), which can progress to pancreatic intraepithelial neoplasia (PanINs) and ultimately adenocarcinoma.[12] These stages of progression of human PDA have been recapitulated in genetically engineered mouse models that target oncogenic *Kras* expression to the pancreas, combined with inactivation of tumor suppressors.[13–15]

PDA is characterized by a dense fibroinflammatory stroma that consists of fibroblasts, vasculature, nerves, extracellular matrix components, and infiltrating immune cells.[16] The immune cells within the tumor microenvironment (TME) are immunosuppressive in nature.[17] Within the TME, there is an extensive infiltration of myeloid cells that directly promote tumor progression[18] and prevent T-cell responses.[19] Accordingly, myeloid cell abundance in tumors correlates with worse outcomes,[20,21] whereas the abundance of tumor-infiltrating T cells correlates with longer survival.[22]

Immune therapy has revolutionized treatment for several malignancies.[23,24] However, the benefit of single agent immunotherapy has not yet extended to PDA,[25,26] with the exception of the 1% of PDA patients with microsatellite instability high tumors.[27] Immune checkpoint therapy acts by reactivating T-cell effector functions most commonly through blockade of programmed cell death 1 (PD-1) or cytotoxic T-lymphocyte antigen 4 (CTLA-4), unleashing anti-tumor T-cell responses that result in reduced tumor burden.[28] Although single agent immunotherapy has not been effective in PDA, recent trials using combination of targeting of T cells and myeloid cells are ongoing, supported by robust preclinical data. In this review, we will describe the critical role myeloid cells play as mediators of immune suppression in PDA and highlight potential strategies to target these cells in the context of combination immunotherapy.

Abbreviations used in this paper: **ADM, acinar to ductal metaplasia; CSFIR, colony-stimulating factor 1 receptor; CTLA-4, cytotoxic T lymphocyte antigen 4; EGFR, epidermal growth factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HB-EGF, heparin-binding EGF-like growth factor; IKK, inhibitory κB kinase; IL, interleukin; MAPK, mitogen-activated protein kinase; MDSC, myeloid-derived suppressor cell; M-MDSC, mononuclear myeloid-derived suppressor cell; NF-κB, nuclear factor kappa B; PanIN, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma; PD-1, programmed cell death; PMN, polymorphonuclear; TAM, tumor-associated macrophage; TME, tumor microenvironment; TNF, tumor necrosis factor.**

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Multiple Myeloid Cell Populations Promote PDA

In normal physiology, myeloid cells develop from hematopoietic stem cells in the bone marrow in a process called myelopoiesis.[29] Myeloid cells are defined as CD45$^+$ CD11b$^+$ cells but further differentiate into distinct populations: macrophages, granulocytes, mast cells, and dendritic cells, all components of the innate immune system. Macrophages within the tumor are referred to as tumor-associated macrophages (TAMs) and have distinct features compared with normal macrophages. Granulocytes can be further divided into eosinophils, basophils, and neutrophils. Within the TME, neutrophils and monocytes are often in an immature state referred to as immature myeloid cells/myeloid-derived suppressor cell (MDSC). In this review we will focus specifically on the role of TAMs and MDSCs in PDA progression (Figure 1).

Tumor-Associated Macrophages

Within the PDA TME, macrophages are an abundant immune cell population.[30,31] Macrophages derived from embryonic progenitors constitute the tissue-resident population; macrophages can also derive from infiltrating monocytes.[32] Macrophages perform multiple physiological functions, including phagocytosis to eliminate debris, antigen presentation, and cytokine secretion to recruit other immune cells to the site of injury.[33,34] Macrophages are defined by expression of CD11b$^+$ CD68$^+$ EMR1$^+$ in humans and CD11b$^+$ CD68$^+$ F4/80$^+$ in mice. Macrophages are plastic cells that exist on a spectrum of differentiation states. On the basis of in vitro assays, macrophages can be classified into 2 main subtypes on each extreme of the spectrum. M1, or classically activated, macrophages are generally considered to have antitumor activities and can be induced through interferon-gamma and toll-like receptor stimuli.[35] M1 macrophages are characterized by high expression of interleukin 12 (IL12), tumor necrosis factor (TNF), and inducible nitric oxide synthase. M2, or alternatively activated, macrophages are considered to have pro-tumor activities[36] and can be induced through the cytokines IL4 and IL13.[37] M2 macrophages lose their antigen presentation abilities and act to instead suppress the immune response through a variety of mechanisms.

The M1/M2 classification is an oversimplification that is helpful for broad description but does not accurately describe the in vivo heterogeneity of TAMs. TAMs within the tumor are derived from either infiltrating monocytes or embryonically derived, tissue-resident macrophages.[38] Furthermore, the heterogeneity of TAM origin has functional implications, where monocyte derived TAMs have increased antigen presentation abilities, and embryonically derived TAMs shape the fibrotic response.[38] Within the TME, TAMs conform to neither the M1 nor the M2 phenotype but rather have traits of both polarization states.[35] Their overall pro-tumor function explains the inverse correlation between TAMs and survival.[39,40]

TAMs have been extensively studied in PDA. Because of the plasticity of macrophages, TAM targeted therapy aims to

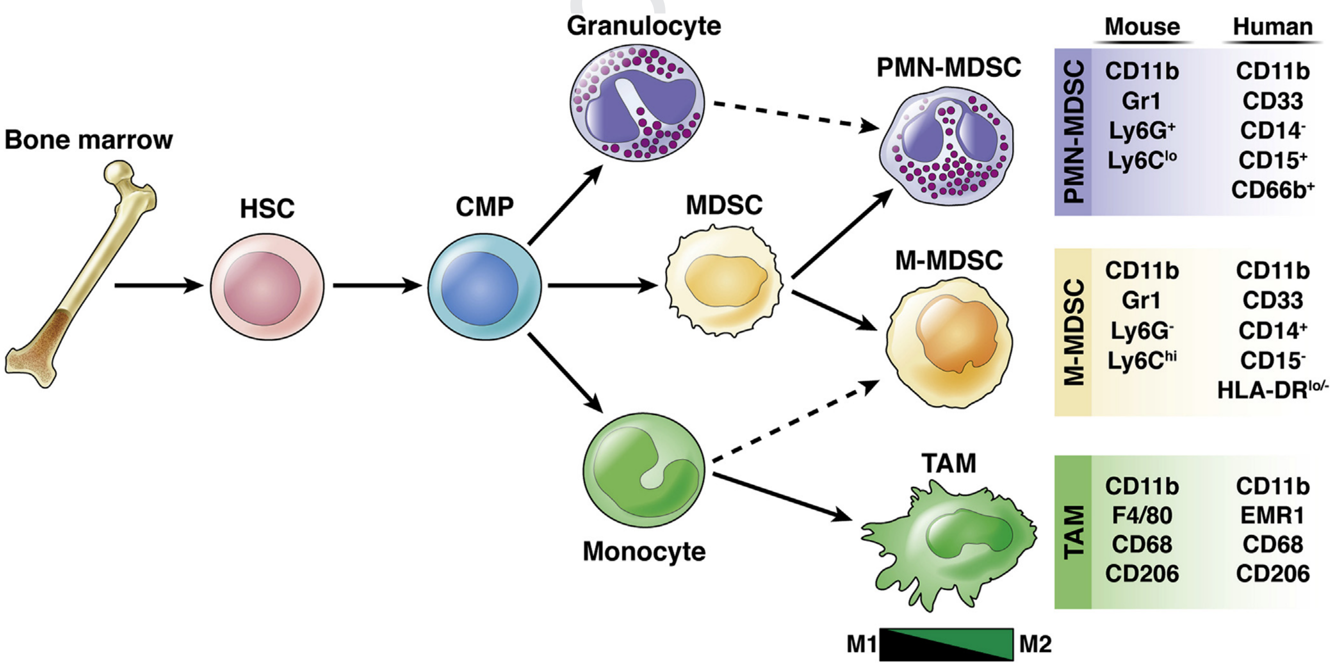

Figure 1. Myeloid cell lineage differentiation and markers. Schematic of myeloid cell differentiation from the bone marrow. Hematopoietic stem cells (HSC) from the bone marrow give rise to common myeloid progenitors (CMP), which give rise to monocytes, granulocytes, and immature myeloid cells, referred to as myeloid-derived suppressor cells (MDSCs). Monocytes in the circulation differentiate into tumor-associated macrophages (TAM) when they enter the tissue. TAMs exist on a spectrum of polarization, with M1 and M2 being at either extreme. MDSCs can be classified into 2 main subsets: PMN-MDSC and M-MDSC. PMN-MDSCs are phenotypically more similar to granulocytes, and M-MDSCs closely resemble monocytes (*dashed arrow*). Surface markers used to define each myeloid population in both mice and humans are listed on the right.

reprogram them to their anti-tumor functions. The colony-stimulating factor 1/colony-stimulating factor 1 receptor (CSF1/CSF1R) axis recruits and polarizes immunosuppressive TAMs. CSF1R is the major lineage regulator for all macrophage subsets.[35] PDA tumors are infiltrated by CSF1R$^+$ macrophages.[41,42] Inhibition of CSF1R in mice results in reduced tumor burden and an increase in T-cell infiltration, providing evidence that targeting TAMs relieves immune suppression in the TME.[19,41] Furthermore, CSF1R inhibition in mice sensitizes PDA tumors to either PD-1 or CTLA-4 antagonists,[42] suggesting that although single agent immunotherapy is not sufficient to reduce tumor burden, immune checkpoint blockade in combination with TAM modulating therapies can effectively reverse immune therapy resistance.

The CCL2/CCR2 chemokine axis is critical for the genesis of TAMs. CCL2 produced by tumor cells recruits CCR2$^+$ monocytes from the bone marrow to the circulation that then differentiate into TAMs after entering the tumor tissue.[43] PDA patients with high levels of circulating monocytes have worse overall survival rates.[20] Monocytes in circulation do not possess the same immunosuppressive abilities as TAMs, suggesting the cellular crosstalk in the TME is critical for this function.[20] CCR2 blockade in mice results in retention of CCR2$^+$ monocytes in the bone marrow, impairing tumor growth.[20] CCR2 blockade in combination with gemcitabine further impairs tumor growth.[20] Similarly, in a PDA clinical trial, patients with borderline resectable and locally advanced disease were treated with a combination of FOLFIRINOX and CCR2 antagonist (PF-04136309).[44] After treatment, patients had reduced circulating CCR2$^+$ monocytes and subsequently fewer TAMs in the tumor, as well as increased CD8$^+$ T cells.[44] However, a recent phase 1b trial evaluated PF-04136309 in combination with gemcitabine/nab-paclitaxel in patients with metastatic PDA.[45] Unlike the previous phase 1b trial, this study did not show that PF-04136309 added additional benefit to the prescribed chemotherapy regimen.[45] Furthermore, in the setting of metastatic PDA, CCR2 inhibition in combination with gemcitabine/nab-paclitaxel was not tolerable in patients.[45] Taken together, these reports suggest that the benefit of CCR2 inhibition may be limited to locally advanced disease that does not extend to metastatic patients.

In addition to an increase in macrophage frequency in PDA, a recent study used multiplex immunofluorescence to evaluate the spatial relationship of M1 and M2 macrophages in human PDA.[46] M1 macrophages were more often found in close proximity to tumor cells, compared with M2 macrophages. Interestingly, when M2 macrophages resided near tumor cells, patients had worse survival outcomes, compared with patients with more distal M2 macrophages. This study provides evidence that both macrophage abundance and location are important factors for patient outcome.

TAMs within the PDA TME express less antigen presenting MHC II,[47] suggesting that macrophages could be reprogrammed to perform their role as antigen presenting cells. CD40 is a member of the TNF receptor superfamily and is expressed broadly on immune cells including monocytes and macrophages.[48,49] Activation of CD40 with an agonist (FGK45) in mice resulted in up-regulation of MHC II in macrophages from the tumor and spleen, suggesting CD40 activation in part reprograms TAMs to an anti-tumor phenotype.[50,51] FGK45 in combination with gemcitabine resulted in reduced tumor burden in a cohort of patients.[50] In addition, combination of gemcitabine and CD40 agonism resulted in increased tumoral T-cell infiltration in mice.[52] Paralleling the human trials, mouse models of PDA are also resistant to single agent immune checkpoint blockade; however, combined chemotherapy and immunotherapy approaches have shown success. Combination therapy of gemcitabine/nab-paclitaxel and aCD40 agonist sensitizes tumors to aPD-1 and aCTLA-4 immunotherapy in murine models of PDA.[53] This combined chemotherapy and immunotherapy approach (gemcitabine, nab-paclitaxel, aCD40 agonist, aPD-1) is currently under clinical trial for patients with metastatic PDA (NCT03214250). Furthermore, in mice, the effectiveness of the combined chemotherapy and immunotherapy regimen can be predicted on the basis of the amount of CD8$^+$ T-cell infiltration, with tumors rich in CD8$^+$ T cells correlating with increased therapeutic response.[54]

Taken together, these studies highlight the tumor promoting role of TAMs in the PDA TME. Macrophage targeted therapy is promising because it synergizes with frontline chemotherapy and immunotherapy regimens to reactivate effector T-cell responses and reduce tumor burden.

Myeloid-Derived Suppressor Cells

MDSCs are immature myeloid cells with immunosuppressive functions. MDSCs can be further classified into 2 main populations, polymorphonuclear (PMN)-MDSCs/granulocytic-MDSCs and mononuclear-MDSCs (M-MDSCs). These subsets are phenotypically distinct. PMN-MDSCs have more resemblance to granulocytes/neutrophils, whereas M-MDSCs closely resemble monocytes. In mice, MDSCs are broadly defined by CD11b$^+$ Gr-1$^+$, with Ly-6C and Ly-6G used to delineate MDSC populations.[55] In mice, MDSCs are defined CD11b$^+$ Ly6C^{lo} Ly6G$^+$ for PMN-MDSCs and CD11b$^+$ Ly6C^{hi} Ly6G$^-$ for M-MDSCs.[55] Because of their phenotypic differences, human PMN-MDSCs, which closely mirror granulocytes/neutrophils, are defined by CD11b$^+$ CD14$^-$ CD15$^+$ or CD11b$^+$ CD14$^-$ CD66b$^+$, whereas human M-MDSCs, which are more similar to monocytes, are defined by CD11b$^+$ CD14$^+$ HLA-DR$^{-/lo}$ CD15$^-$.[55] Although PMN-MDSCs and M-MDSCs are the major MDSC populations, there are MDSCs that share markers of both and may represent a common progenitor. This third MDSC population is called early stage MDSCs and has yet to be functionally evaluated in PDA.[55] Although MDSCs are unique from their mature myeloid counterparts, neutrophils and monocytes, controversy remains on separating PMN-MDSCs from neutrophils. Currently, there are no markers to distinguish the immature PMN-MDSCs from mature neutrophils, and the only possible method of separation is via density centrifugation.[56] M-MDSCs differ from monocytes because they express low HLA-DR and differ from TAMs because they do not express F4/80.[57] Distinction between neutrophils and PMN-MDSCs remains challenging, and distinctive markers are needed.

Importantly, MDSCs are ultimately defined by their functionality. MDSCs perform their immune suppressive functions through multiple mechanisms, with the main one being depletion of the essential amino acid L-arginine from the TME.[58,59] MDSCs produce high levels of Arginase 1 (ARG1), an enzyme that metabolizes L-arginine, resulting in T-cell inhibition.[60] When considering MDSC function, it is important to also consider that MDSCs exist in 2 main populations. PMN-MDSCs comprise the largest percentage of MDSCs found in the blood and the tumor, compared with M-MDSCs.[61] Despite M-MDSCs making up a smaller portion of the tumor, they often have an increased immunosuppressive function than PMN-MDSCs.[62] Both MDSC populations express high amounts of the enzyme ARG1, which depletes L-arginine, resulting in T-cell inhibition.[63] However, PMN-MDSCs and M-MDSCs have additional and distinct immunosuppressive functions. PMN-MDSCs produce high amounts of reactive oxygen species and low nitric oxide.[61] M-MDSCs produce high nitric oxide and low reactive oxygen species.[61] Furthermore, M-MDSC immune suppression is in part due to tumor cell-derived prostaglandin E2 activating p50, a nuclear factor kappa B (NF-κB) subunit that results in increased inducible nitric oxide synthase production.[64] These data show MDSC populations have distinct mechanisms to suppress T cells.

Because of the immunosuppressive nature of MDSCs, targeting these cells within the PDA TME is an attractive option for pancreatic cancer treatment. Early work in mouse models targeted MDSCs through administration of zoledronic acid, which acts to reduce MDSCs recruitment through inhibition of matrix metalloproteinase 9.[65] Administration of zoledronic acid in a PDA mouse model results in delayed tumor growth, enhanced survival, and increased CD8$^+$ T-cell infiltration.[66] CXCR2 is a receptor found on neutrophils/MDSCs and regulates the recruitment of MDSCs to the TME.[67] Inhibition of CXCR2 in a genetically engineered mouse model of pancreatic cancer resulted in extended survival, an increase in T-cell infiltration, and synergy with immunotherapy.[68] MDSCs are also recruited to the tumor through tumor cell-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion. Neutralization of GM-CSF in murine models of PDA results in a reduction in MDSC recruitment and subsequently reduced tumor growth.[69,70] Depletion of the PMN-MDSC subset with an antibody against Ly-6G results in tumor cell death and increased CD8$^+$ T-cell infiltration.[71] Thus, MDSC-targeted therapies can partially reverse immune suppression.

Myeloid-Epithelial Crosstalk Promotes Immune Suppression

Myeloid cells do not act alone in establishing an immune suppressive TME. Rather, they act as a central hub in a complex cellular crosstalk that promotes tumor progression. Here we will explore mechanisms of cellular crosstalk between myeloid cells and cancer cells that activate signaling pathways that enhance immune suppression (Figure 2).

Beyond their role in establishing an immunosuppressive TME, myeloid cells play a critical role in promoting pancreatic carcinogenesis.[18,72–74] In a PDA mouse model driven by inducible expression of oncogenic *Kras*^{G12D} (iKras),[75] myeloid cell ablation––using CD11b promoter driven expression of the diphtheria toxin receptor followed by diphtheria toxin treatment[76]–– causes regression of early PanIN lesions, preceded by reduced ERK activity in the neoplasia.[18] Although oncogenic *KRAS* is the main genetic driver of PDA, it is not sufficient to induce carcinogenesis without additional activation of epidermal growth factor receptor (EGFR) to amplify mitogen-activated protein kinase (MAPK) signaling in the epithelium.[77,78] Of note, myeloid cells in the neoplastic pancreas express high levels of the EGFR ligands, heparin-binding EGF-like growth factor (HB-EGF) and epiregulin, suggesting that they promote the initial stages of pancreatic carcinogenesis by stimulating epithelial EGFR. Conversely, oncogenic *Kras* expression in the epithelium also alters macrophage polarization.[18] Extinguishing *Kras* expression in the iKras model results in decreased expression of Arginase 1 (*Arg1*) and the EGFR ligand HB-EGF (*Hbegf*) in the myeloid compartment, with

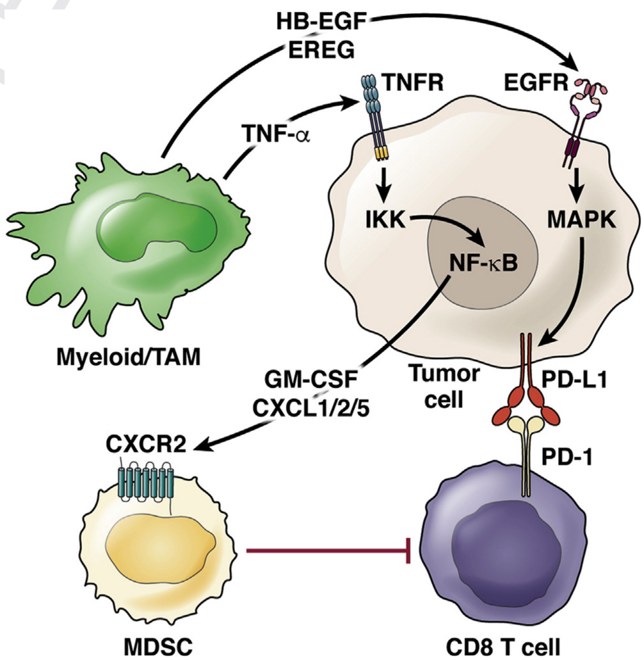

Figure 2. Myeloid-epithelial crosstalk promotes immune suppression. Schematic for cellular crosstalk and corresponding signaling pathways in the PDA TME that contribute to immune suppression. Myeloid cells secrete various ligands, HB-EGF, EREG, and TNF-α, that signal to their respective receptors, EGFR and TNFR, on tumor cells, thus activating EGFR/MAPK and NF-κB signaling, respectively. MAPK signaling in tumor cells results in elevation of PD-L1 expression, inhibiting CD8$^+$ T cells through interaction with PD-1. NF-κB signaling in tumor cells results in secretion of GM-CSF and CXCL1, CXCL2, and CXCL5, which recruit MDSCs with the potential to suppress CD8$^+$ T cells.

subsequent loss of EGFR (*Egfr*) expression in the epithelial compartment. These data suggest that KRAS/EGFR/MAPK signaling regulates myeloid cell infiltration and polarization before PanIN formation, which in turn promotes epithelial transformation and progression of the neoplasia.

In addition to its early role in PDA formation, EGFR also regulates immune suppression in mouse models after carcinogenesis.[74,79] Myeloid cell ablation from preexisting tumors results in reduced tumor burden, providing evidence that myeloid cells drive carcinogenesis in both early and late stages of disease.[74] Myeloid cells secrete HB-EGF, an EGFR ligand, which activates EGFR/MAPK signaling in tumor cells leading to increased PD-L1 expression.[74] Furthermore, ablation of EGFR in PDA sensitized tumors to chemotherapy and immunotherapy.[79] Treatment with the EGFR inhibitor erlotinib reduced tumoral myeloid cells, increased $CD8^+$ T cells, and enhanced response to immunotherapy.[79] These studies suggest a role for EGFR/MAPK in promoting carcinogenesis and myeloid-mediated immune suppression.

NF-κB is a transcription factor with known diverse function in regulation of the immune system.[80] Dysregulated NF-κB signaling can lead to inflammatory conditions such as cancer.[81] Along with *KRAS*, NF-κB is constitutively active in PDA patients.[82,83] NF-κB is held inactive in the cytoplasm in a complex with inhibitory κB proteins. Extracellular signals, such as TNFR ligation, activate inhibitory κB kinase (IKK), phosphorylate inhibitory κB, targeting it for degradation and resulting in the nuclear translocation of NF-κB complexes to activate transcription of target genes. The IKK complex is made up of 2 kinases, IKKa and IKKb, and an additional subunit, NEMO/IKKg.[84] Inactivation of IKKb in PDA tumors reduced infiltration of macrophages and MDSCs and blocked carcinogenesis, extending survival.[82] Having established that both macrophages and NF-κB are important for initial transformation, it is interesting to note that one study linked an enhancement of ADM, the initial step of transformation, to macrophage production of TNF and subsequent activation of NF-κB.[73] These data suggest NF-κB is not only critical for PDA formation but also mediates myeloid cell infiltration in the tumor.

NF-κB signaling also activates GM-CSF secretion.[85] GM-CSF is a cytokine that functions to recruit MDSCs.[69,70] Human PDA tumor cells treated with chemotherapy (gemcitabine or 5-FU) have increased levels of GM-CSF.[86] Coincidentally, human tumor cells treated with gemcitabine have increased NF-κB activity. Monocytes cultured with chemotherapy treated tumor cells promote differentiation into immunosuppressive MDSCs.[86] Taken together, these data suggest one possible mechanism for chemoresistance in PDA is active NF-κB signaling in tumor cells, which promotes an immunosuppressive myeloid phenotype, exacerbating disease.

NF-κB activates the expression of the chemokines CXCL1, CXCL2, and CXCL5, which in turn recruit $CXCR2^+$ MDSCs, resulting in T-cell suppression.[87–89] PDA patients have a heterogenous infiltration of T cells.[90,91] Recent work identified CXCL1 as one mediator for T-cell heterogeneity in the PDA TME.[54] Overexpression of tumor cell-derived *Cxcl1* increases myeloid infiltration, specifically the granulocytic MDSCs, and fewer infiltrating $CD8^+$ T cells, providing further evidence on the immunosuppressive role of CXCL1 in the TME.[54] Furthermore, ablation of *Cxcl1* in tumor cells results in fewer granulocytic MDSCs and a subsequent increase in $CD8^+$ T cells, allowing the tumors to be sensitized to immunotherapy.[54]

Clearly, there is a complex cellular crosstalk between tumor cells and myeloid cells that suppresses T-cell infiltration and function in the TME. Multiple pathways are implicated in this immune suppressive phenotype. Work thus far targeting this tumor-myeloid interaction is compelling because it sensitizes tumors to immunotherapy approaches, highlighting the translational implications for PDA patients.

Myeloid Cells Establish the Pre-Metastatic Niche and Promote Metastatic Disease

The majority of PDA patients present with metastatic disease, and for those patients, limited therapeutic options are available. The liver is the most common site for metastatic dissemination in PDA. Pancreatic tumor cells disseminate early in carcinogenesis before progression to carcinoma.[92] Despite the severity of metastatic disease, the process of metastasis is inefficient.[93] A key barrier to tumor cell dissemination and survival in distal organs is the requirement of support from stromal cells.[94] Inflammation is critical for progression of the primary tumor[95] but is also critical for tumor cell dissemination.[92] Myeloid cells colonize these distal sites before the arrival of the tumor cells in principle to create a hospitable environment for tumor cell growth[96–99] in a concept termed the pre-metastatic niche.

Currently, few studies have been performed evaluating the pre-metastatic niche in PDA. One study showed macrophages that are recruited to the liver secrete granulin, which in turn activates myofibroblasts, creating a permissive environment for tumor cell survival.[94] Exosomes from tumor cells were identified as another mediator that promotes formation of the liver pre-metastatic niche in PDA.[100] Tumor derived exosomes are taken up by Kupffer cells, resident liver macrophages, resulting in increased fibrosis in the liver and increased macrophage accumulation.[100] This stromal accumulation prepares the liver for ultimate tumor cell survival. Macrophage migration inhibitory factor was determined to be the primary exosome cargo driving the pre-metastatic niche formation. As such, macrophage migration inhibitory factor ablation prevented formation of the pre-metastatic niche and subsequently reduced liver metastasis.[100]

IL6/signal transducer and activator of transcription 3/serum amyloid A signaling is another critical mechanism for the formation of the liver pre-metastatic niche.[97] Rather than tumor cell-mediated formation of the pre-metastatic niche, this study identifies hepatocytes as an additional driver of the pre-metastatic niche.[97] Genetic ablation of individual components of IL6/signal transducer and activator of transcription 3/serum amyloid A signaling resulted in fewer macrophages and PMN-MDSCs (Ly-$6G^+$), preventing metastatic dissemination. The concept of the pre-metastatic niche is an important question that is relatively unexplored in PDA. Each of these studies provides a framework to explain the role myeloid cells play in pre-metastatic formation. Thus, identifying methods to interfere with myeloid function has the potential to mitigate metastasis of this highly aggressive cancer.

In addition to their role in tumorigenesis and pre-metastatic niche preparation, myeloid cells have been implicated in migration and invasion of metastatic disease in many cancer types.[35,101,102] CCR2[20] and CXCR2[68] inhibition reduces metastatic dissemination in PDA through ablation of monocytes/macrophages and MDSCs, respectively. MDSC depletion in mouse PDA tumors converts the tumor from the highly invasive basal subtype to the less aggressive classical subtype and extended survival.[68,103] Furthermore, pharmacologic depletion of macrophages with liposomal clodronate impairs angiogenesis and reduces metastasis formation in mice with PDA.[104] Myeloid cells appear to be critical for both the formation of the pre-metastatic niche and metastatic dissemination.

Macrophages Drive Resistance to Chemotherapy

Because immune therapy has been ineffective in treating PDA, frontline therapy remains chemotherapy regimens, although they have only marginal efficacy.[4,6,105,106] Current standard-of-care chemotherapy regimens for PDA patients include gemcitabine/nab-paclitaxel and FOLFIRINOX. However, PDA tumors are highly chemoresistant. A broad approach of depleting all myeloid cells using CD11b-DTR mice treated with diphtheria toxin results in tumors being sensitized to gemcitabine,[107] suggesting myeloid cells can be targeted to reverse chemoresistance. Furthermore, dual inhibition of TAMs ($CCR2^+$) and MDSCs ($CXCR2^+$) resulted in increased efficacy of FOLFIRINOX.[108]

Myeloid Cell Compensatory Responses

Throughout this review we have highlighted a myriad of reports targeting monocytes/macrophages and MDSCs in PDA. It has become clear that these approaches, while beneficial, often result in a compensatory response of the other myeloid cell subsets. Two studies in PDA report a compensatory increase in monocyte and macrophage subsets when MDSCs are depleted.[71,108] To prevent compensatory myeloid infiltration, another approach is to target all myeloid cells via integrin CD11b on their surface. Although antagonists for CD11b exist,[109,110] they have not been well-tolerated in patients because of toxicity.[111] Instead, an alternative approach to activate CD11b rather than antagonize has shown promise in preventing inflammation.[112] The small molecule CD11b agonist reduces inflammation in a mouse model of PDA.[113] CD11b agonism reduces myeloid infiltration, increases T-cell infiltration, and sensitizes tumors to both chemotherapy and immunotherapy.[113] Although the total number of myeloid cells was reduced with CD11b agonism, macrophages that remained were reprogrammed, reducing the expression of a number of immunosuppressive genes (expressing Arginase 1, IL10, transforming growth factor beta) and increasing antigen presentation abilities, leading to activation of classical dendritic cells and subsequent T-cell infiltration.[113] CD11b agonism is one potential avenue to avoid myeloid cell compensation when targeting a select myeloid cell subset.

Myeloid cells compensate for depletion of regulatory T cells, another immunosuppressive cell type in the PDA TME.[114] In one study, depletion of regulatory T cells did not reverse immune suppression as hypothesized but rather accelerated tumor progression, in part because of a compensatory infiltration of immunosuppressive myeloid cells (Arginase 1, Chitinase3-like-3/YM1). This sustained immunosuppression was reduced through inhibition of the myeloid receptor CCR1, providing further indication that myeloid cells promote tumor progression and have complex and compensatory roles in the PDA TME.

Myeloid Single Cell Transcriptomics

Recent single cell RNA sequencing efforts in PDA have revealed significant heterogeneity within myeloid cell subsets that confirm the M1/M2 designation is an oversimplification. Analysis of human PDA tumor samples compared with adjacent normal pancreas tissue identified populations of neutrophils, classical monocytes/macrophages, resident macrophages, and alternatively activated macrophages.[115] *MARCO*, *APOE*, *SPP1*, and *C1QA* emerged as novel macrophage markers that warrant further evaluation in PDA.[115] Another study identified similar myeloid populations in human PDA compared with adjacent normal pancreas tissue with similar gene expression profiles.[116] Myeloid cells are shown to have heterogenous expression of immune checkpoint receptors (*LGALS9*, *CD274*, *PVR*, *CSF1R*, *SIRPA*, *HLA-DQA1*).[116] Putative immune checkpoint interactions were up-regulated in PDA compared with adjacent normal samples, and these interactions were heterogenous across patients.[116] Because of the overwhelming lack of response to immunotherapy approaches, these data suggest the heterogeneity of immune checkpoints across patients is a contributing factor, and we should consider the possibility of precision medicine in immunomodulatory approaches.

Two studies used single cell transcriptomics analysis to evaluate the immune response during mouse PDA progression.[117,118] Consistent with previous reports, macrophages were identified as one of the major immune cells infiltrating early lesions. Through unbiased clustering, 3 macrophage populations were identified in early lesions, whereas only 2 macrophage populations were identified in late/tumor samples.[118] The macrophage population only found in early lesion samples had expression of *Fn1*, *Lyz1*, and *Ear1*, suggesting this population is involved in wound repair.[118] There was not an equivalent macrophage population to this one seen in the late-stage tumor samples, suggesting macrophage populations change over the course of disease progression. In a separate study, macrophages from late lesions compared with early lesion samples had an increase in the chemokines, *Cxcl1*, *Cxcl2*, and *Ccl8,* which have known roles in recruitment of MDSCs (*Cxcl1*, *Cxcl2*) and macrophages (*Ccl8*), suggesting sustained infiltration of myeloid cells as carcinogenesis progresses.[117] These macrophages up-regulated markers of alternative activation (*Mrc1*), further supporting the concept that macrophage polarization changes in later stages of PDA. Importantly, these combined efforts have revealed novel myeloid cells markers with potential functional importance in PDA.

Conclusions and Future Directions

In this review we have defined myeloid cell subsets in the PDA TME and discussed their role in myeloid cell-mediated immune suppression. We highlight the importance of myeloid cells through disease progression from initial formation of ADM to carcinogenesis to the formation of the pre-metastatic niche leading to ultimate tumor cell dissemination. Current myeloid targeted approaches in combination with chemotherapy and immunotherapy regimens relieve this robust immune suppression and activate T-cell effector responses.

However, many questions remain unanswered. The mechanisms behind the inverse correlation of myeloid cell and T cells have yet to be fully elucidated. Although we have some understanding of the pathways involved, we are lacking the complete picture, especially with respect to the complex compensatory networks that appear to overcome monolithic approaches. A better understanding of the mechanisms behind myeloid-mediated immune suppression will uncover novel and hopefully targetable components. With the large influx of single cell transcriptomics data, it has become even more evident that the M1/M2 designation is a gross oversimplification and does not accurately mirror the in vivo heterogeneity of macrophages. These reports have uncovered novel macrophage markers that may have functional implications and should be evaluated. Most of the MDSC work in PDA has targeted the PMN-MDSC subset. Because the M-MDSCs are more immunosuppressive in nature, selectively targeting this cell population is of interest. Myeloid cells comprise the largest part of the TME and are ideal targets to reverse immune suppression.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7–30.
2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913–2921.
3. Kleeff J, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin AV, Neale RE, Tempero M, Tuveson DA, Hruban RH, Neoptolemos JP. Pancreatic cancer. Nat Rev Dis Primers 2016;2:16022.
4. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, Bennouna J, Bachet JB, Khemissa-Akouz F, Pere-Verge D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M, Groupe Tumeurs Digestives of U, Intergroup P. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817–1825.
5. Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Chone L, Francois E, Artru P, Biagi JJ, Lecomte T, Assenat E, Faroux R, Ychou M, Volet J, Sauvanet A, Breysacher G, Di Fiore F, Cripps C, Kavan P, Texereau P, Bouhier-Leporrier K, Khemissa-Akouz F, Legoux JL, Juzyna B, Gourgou S, O'Callaghan CJ, Jouffroy-Zeller C, Rat P, Malka D, Castan F, Bachet JB. Canadian Cancer Trials G, the Unicancer GIPG. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med 2018;379:2395–2406.
6. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691–1703.
7. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 1988;53:549–554.
8. Hata T, Suenaga M, Marchionni L, Macgregor-Das A, Yu J, Shindo K, Tamura K, Hruban RH, Goggins M. Genome-wide somatic copy number alterations and mutations in high-grade pancreatic intraepithelial neoplasia. Am J Pathol 2018;188:1723–1733.
9. Maitra A, Hruban RH. Pancreatic cancer. Annu Rev Pathol 2008;3:157–188.
10. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 2006;20:1218–1249.
11. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JPt, Pan FC, Akiyama H, Wright CV, Jensen K, Hebrok M, Sander M. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell 2012;22:737–750.
12. Storz P. Acinar cell plasticity and development of pancreatic ductal adenocarcinoma. Nat Rev Gastroenterol Hepatol 2017;14:296–304.
13. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 2003; 17:3112–3126.
14. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003;4:437–450.
15. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic

pancreatic ductal adenocarcinoma in mice. Cancer Cell 2005;7:469–483.

16. Chu GC, Kimmelman AC, Hezel AF, DePinho RA. Stromal biology of pancreatic cancer. J Cell Biochem 2007; 101:887–907.
17. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res 2007;67:9518–9527.
18. Zhang Y, Yan W, Mathew E, Kane KT, Brannon A 3rd, Adoumie M, Vinta A, Crawford HC, Pasca di Magliano M. Epithelial-myeloid cell crosstalk regulates acinar cell plasticity and pancreatic remodeling in mice. Elife 2017;6.
19. Mitchem JB, Brennan DJ, Knolhoff BL, Belt BA, Zhu Y, Sanford DE, Belaygorod L, Carpenter D, Collins L, Piwnica-Worms D, Hewitt S, Udupi GM, Gallagher WM, Wegner C, West BL, Wang-Gillam A, Goedegebuure P, Linehan DC, DeNardo DG. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res 2013;73:1128–1141.
20. Sanford DE, Belt BA, Panni RZ, Mayer A, Deshpande AD, Carpenter D, Mitchem JB, Plambeck-Suess SM, Worley LA, Goetz BD, Wang-Gillam A, Eberlein TJ, Denardo DG, Goedegebuure SP, Linehan DC. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/ CCR2 axis. Clin Cancer Res 2013;19:3404–3415.
21. Tsujikawa T, Kumar S, Borkar RN, Azimi V, Thibault G, Chang YH, Balter A, Kawashima R, Choe G, Sauer D, El Rassi E, Clayburgh DR, Kulesz-Martin MF, Lutz ER, Zheng L, Jaffee EM, Leyshock P, Margolin AA, Mori M, Gray JW, Flint PW, Coussens LM. Quantitative multiplex immunohistochemistry reveals myeloid-inflamed tumor-immune complexity associated with poor prognosis. Cell Rep 2017;19:203–217.
22. Balachandran VP, Luksza M, Zhao JN, Makarov V, Moral JA, Remark R, Herbst B, Askan G, Bhanot U, Senbabaoglu Y, Wells DK, Cary CIO, Grbovic-Huezo O, Attiyeh M, Medina B, Zhang J, Loo J, Saglimbeni J, Abu-Akeel M, Zappasodi R, Riaz N, Smoragiewicz M, Kelley ZL, Basturk O, Australian Pancreatic Cancer Genome I; , Garvan Institute of Medical R; , Prince of Wales H; , Royal North Shore H; , University of G, St Vincent's H; , Institute QBMR; , University of Melbourne CfCR; , University of Queensland IfMB; , Bankstown H, Liverpool H, Royal Prince Alfred Hospital COBL, Westmead H, Fremantle H, St John of God H, Royal Adelaide H, Flinders Medical C, Envoi P, Princess Alexandria H, Austin H, Hopkins Johns, Medical I, Cancer AR-NCfARo, Gonen M, Levine AJ, Allen PJ, Fearon DT, Merad M, Gnjatic S, Iacobuzio-Donahue CA, Wolchok JD, DeMatteo RP, Chan TA, Greenbaum BD, Merghoub T, Leach SD. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature 2017;551:512–516.
23. Weiss SA, Wolchok JD, Sznol M. Immunotherapy of melanoma: facts and hopes. Clin Cancer Res 2019; 25:5191–5201.
24. Doroshow DB, Sanmamed MF, Hastings K, Politi K, Rimm DL, Chen L, Melero I, Schalper KA, Herbst RS. Immunotherapy in non-small cell lung cancer: facts and hopes. Clin Cancer Res 2019;25:4592–4602.
25. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455–2465.
26. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, Rosenberg SA. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother 2010; 33:828–833.
27. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA Jr. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–413.
28. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol 2020;20:651–668.
29. Messmer MN, Netherby CS, Banik D, Abrams SI. Tumor-induced myeloid dysfunction and its implications for cancer immunotherapy. Cancer Immunol Immunother 2015;64:1–13.
30. Long KB, Collier AI, Beatty GL. Macrophages: key orchestrators of a tumor microenvironment defined by therapeutic resistance. Mol Immunol 2019;110:3–12.
31. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol 2019;19:369–382.
32. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013; 496:445–455.
33. Watanabe S, Alexander M, Misharin AV, Budinger GRS. The role of macrophages in the resolution of inflammation. J Clin Invest 2019;129:2619–2628.
34. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 2011; 11:723–737.
35. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010;141:39–51.
36. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–555.
37. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3:23–35.

38. Zhu Y, Herndon JM, Sojka DK, Kim KW, Knolhoff BL, Zuo C, Cullinan DR, Luo J, Bearden AR, Lavine KJ, Yokoyama WM, Hawkins WG, Fields RC, Randolph GJ, DeNardo DG. Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. Immunity 2017;47:323–338 e6.
39. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, Sakoda M, Ueno S, Natsugoe S, Takao S. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res 2011; 167:e211–e219.
40. Ino Y, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, Hiraoka N. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer 2013;108:914–923.
41. Candido JB, Morton JP, Bailey P, Campbell AD, Karim SA, Jamieson T, Lapienyte L, Gopinathan A, Clark W, McGhee EJ, Wang J, Escorcio-Correia M, Zollinger R, Roshani R, Drew L, Rishi L, Arkell R, Evans TRJ, Nixon C, Jodrell DI, Wilkinson RW, Biankin AV, Barry ST, Balkwill FR, Sansom OJ. CSF1R(+) macrophages sustain pancreatic tumor growth through T cell suppression and maintenance of key gene programs that define the squamous subtype. Cell Rep 2018;23:1448–1460.
42. Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, Wang-Gillam A, Goedegebuure SP, Linehan DC, DeNardo DG. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014;74:5057–5069.
43. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol 2011;11:762–774.
44. Nywening TM, Wang-Gillam A, Sanford DE, Belt BA, Panni RZ, Cusworth BM, Toriola AT, Nieman RK, Worley LA, Yano M, Fowler KJ, Lockhart AC, Suresh R, Tan BR, Lim KH, Fields RC, Strasberg SM, Hawkins WG, DeNardo DG, Goedegebuure SP, Linehan DC. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial. Lancet Oncol 2016;17:651–662.
45. Noel M, O'Reilly EM, Wolpin BM, Ryan DP, Bullock AJ, Britten CD, Linehan DC, Belt BA, Gamelin EC, Ganguly B, Yin D, Joh T, Jacobs IA, Taylor CT, Lowery MA. Phase 1b study of a small molecule antagonist of human chemokine (C-C motif) receptor 2 (PF-04136309) in combination with nab-paclitaxel/gemcitabine in first-line treatment of metastatic pancreatic ductal adenocarcinoma. Invest New Drugs 2020;38:800–811.
46. Vayrynen SA, Zhang J, Yuan C, Vayrynen JP, Dias Costa A, Williams H, Morales-Oyarvide V, Lau MC, Rubinson DA, Dunne RF, Kozak MM, Wang W, Agostini-Vulaj D, Drage MG, Brais L, Reilly E, Rahma O, Clancy T, Wang J, Linehan DC, Aguirre AJ, Fuchs CS, Coussens LM, Chang DT, Koong AC, Hezel AF, Ogino S, Nowak JA, Wolpin BM. Composition, spatial characteristics, and prognostic significance of myeloid cell infiltration in pancreatic cancer. Clin Cancer Res 2021;27:1069–1081.
47. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–1570.
48. Vonderheide RH. Prospect of targeting the CD40 pathway for cancer therapy. Clin Cancer Res 2007; 13:1083–1088.
49. Vonderheide RH, Bajor DL, Winograd R, Evans RA, Bayne LJ, Beatty GL. CD40 immunotherapy for pancreatic cancer. Cancer Immunol Immunother 2013;62:949–954.
50. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL, Torigian DA, O'Dwyer PJ, Vonderheide RH. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science 2011;331:1612–1616.
51. Beatty GL. Macrophage-based immunotherapy for the treatment of pancreatic ductal adenocarcinoma. Oncoimmunology 2013;2:e26837.
52. Beatty GL, Winograd R, Evans RA, Long KB, Luque SL, Lee JW, Clendenin C, Gladney WL, Knoblock DM, Guirnalda PD, Vonderheide RH. Exclusion of T cells from pancreatic carcinomas in mice is regulated by Ly6C(low) F4/80(+) extratumoral macrophages. Gastroenterology 2015;149:201–210.
53. Winograd R, Byrne KT, Evans RA, Odorizzi PM, Meyer AR, Bajor DL, Clendenin C, Stanger BZ, Furth EE, Wherry EJ, Vonderheide RH. Induction of T-cell immunity overcomes complete resistance to PD-1 and CTLA-4 blockade and improves survival in pancreatic carcinoma. Cancer Immunol Res 2015;3:399–411.
54. Li J, Byrne KT, Yan F, Yamazoe T, Chen Z, Baslan T, Richman LP, Lin JH, Sun YH, Rech AJ, Balli D, Hay CA, Sela Y, Merrell AJ, Liudahl SM, Gordon N, Norgard RJ, Yuan S, Yu S, Chao T, Ye S, Eisinger-Mathason TSK, Faryabi RB, Tobias JW, Lowe SW, Coussens LM, Wherry EJ, Vonderheide RH, Stanger BZ. Tumor cell-intrinsic factors underlie heterogeneity of immune cell infiltration and response to immunotherapy. Immunity 2018;49:178–193 e7.
55. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH, Gabrilovich DI. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 2016;7:12150.
56. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. J Clin Invest 2015;125:3356–3364.
57. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. Nat Immunol 2018; 19:108–119.
58. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–174.
59. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 2005; 5:641–654.

60. Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunol Rev 2008;222:180–191.
61. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008;181:5791–5802.
62. Trovato R, Fiore A, Sartori S, Cane S, Giugno R, Cascione L, Paiella S, Salvia R, De Sanctis F, Poffe O, Anselmi C, Hofer F, Sartoris S, Piro G, Carbone C, Corbo V, Lawlor R, Solito S, Pinton L, Mandruzzato S, Bassi C, Scarpa A, Bronte V, Ugel S. Immunosuppression by monocytic myeloid-derived suppressor cells in patients with pancreatic ductal carcinoma is orchestrated by STAT3. J Immunother Cancer 2019;7:255.
63. Raber P, Ochoa AC, Rodriguez PC. Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell suppression and therapeutic perspectives. Immunol Invest 2012;41:614–634.
64. Porta C, Consonni FM, Morlacchi S, Sangaletti S, Bleve A, Totaro MG, Larghi P, Rimoldi M, Tripodo C, Strauss L, Banfi S, Storto M, Pressiani T, Rimassa L, Tartari S, Ippolito A, Doni A, Solda G, Duga S, Piccolo V, Ostuni R, Natoli G, Bronte V, Balzac F, Turco E, Hirsch E, Colombo MP, Sica A. Tumor-derived prostaglandin E2 promotes p50 NF-kappaB-dependent differentiation of monocytic MDSCs. Cancer Res 2020; 80:2874–2888.
65. Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. Cancer Res 2007;67:11438–11446.
66. Porembka MR, Mitchem JB, Belt BA, Hsieh CS, Lee HM, Herndon J, Gillanders WE, Linehan DC, Goedegebuure P. Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth. Cancer Immunol Immunother 2012;61:1373–1385.
67. Highfill SL, Cui Y, Giles AJ, Smith JP, Zhang H, Morse E, Kaplan RN, Mackall CL. Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy. Sci Transl Med 2014;6:237ra67.
68. Steele CW, Karim SA, Leach JDG, Bailey P, Upstill-Goddard R, Rishi L, Foth M, Bryson S, McDaid K, Wilson Z, Eberlein C, Candido JB, Clarke M, Nixon C, Connelly J, Jamieson N, Carter CR, Balkwill F, Chang DK, Evans TRJ, Strathdee D, Biankin AV, Nibbs RJB, Barry ST, Sansom OJ, Morton JP. CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. Cancer Cell 2016;29:832–845.
69. Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, Vonderheide RH. Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. Cancer Cell 2012;21:822–835.
70. Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G, Bar-Sagi D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell 2012;21:836–847.
71. Stromnes IM, Brockenbrough JS, Izeradjene K, Carlson MA, Cuevas C, Simmons RM, Greenberg PD, Hingorani SR. Targeted depletion of an MDSC subset unmasks pancreatic ductal adenocarcinoma to adaptive immunity. Gut 2014;63:1769–1781.
72. Liou GY, Doppler H, Necela B, Edenfield B, Zhang L, Dawson DW, Storz P. Mutant KRAS-induced expression of ICAM-1 in pancreatic acinar cells causes attraction of macrophages to expedite the formation of precancerous lesions. Cancer Discov 2015;5:52–63.
73. Liou GY, Doppler H, Necela B, Krishna M, Crawford HC, Raimondo M, Storz P. Macrophage-secreted cytokines drive pancreatic acinar-to-ductal metaplasia through NF-kappaB and MMPs. J Cell Biol 2013;202:563–577.
74. Zhang Y, Velez-Delgado A, Mathew E, Li D, Mendez FM, Flannagan K, Rhim AD, Simeone DM, Beatty GL, Pasca di Magliano M. Myeloid cells are required for PD-1/PD-L1 checkpoint activation and the establishment of an immunosuppressive environment in pancreatic cancer. Gut 2017;66:124–136.
75. Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, Galban CJ, Rakshit S, Flannagan KS, Adsay NV, Pasca di Magliano M. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest 2012;122:639–653.
76. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56–65.
77. Ardito CM, Gruner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, Delgiorno KE, Carpenter ES, Halbrook CJ, Hall JC, Pal D, Briel T, Herner A, Trajkovic-Arsic M, Sipos B, Liou GY, Storz P, Murray NR, Threadgill DW, Sibilia M, Washington MK, Wilson CL, Schmid RM, Raines EW, Crawford HC, Siveke JT. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. Cancer Cell 2012;22:304–317.
78. Collins MA, Yan W, Sebolt-Leopold JS, Pasca di Magliano M. MAPK signaling is required for dedifferentiation of acinar cells and development of pancreatic intraepithelial neoplasia in mice. Gastroenterology 2014; 146:822–834 e7.
79. Li J, Yuan S, Norgard RJ, Yan F, Sun YH, Kim IK, Merrell AJ, Sela Y, Jiang Y, Bhanu NV, Garcia BA, Vonderheide RH, Blanco A, Stanger BZ. Epigenetic and transcriptional control of the epidermal growth factor receptor (EGFR) regulates the tumor immune microenvironment in pancreatic cancer. Cancer Discov 2020.
80. Zhang Q, Lenardo MJ, Baltimore D. 30 years of NF-kappaB: a blossoming of relevance to human pathobiology. Cell 2017;168:37–57.
81. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 2006;25:6680–6684.
82. Ling J, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z, Li J, Peng B, Fleming JB, Wang H, Liu J, Lemischka IR, Hung MC, Chiao PJ. KrasG12D-induced IKK2/beta/NF-kappaB activation by IL-1alpha and p62 feedforward

loops is required for development of pancreatic ductal adenocarcinoma. Cancer Cell 2012;21:105–120.

83. Maier HJ, Wagner M, Schips TG, Salem HH, Baumann B, Wirth T. Requirement of NEMO/IKKgamma for effective expansion of KRAS-induced precancerous lesions in the pancreas. Oncogene 2013;32:2690–2695.

84. Israel A. The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harb Perspect Biol 2010;2:a000158.

85. Schreck R, Baeuerle PA. NF-kappa B as inducible transcriptional activator of the granulocyte-macrophage colony-stimulating factor gene. Mol Cell Biol 1990; 10:1281–1286.

86. Takeuchi S, Baghdadi M, Tsuchikawa T, Wada H, Nakamura T, Abe H, Nakanishi S, Usui Y, Higuchi K, Takahashi M, Inoko K, Sato S, Takano H, Shichinohe T, Seino K, Hirano S. Chemotherapy-derived inflammatory responses accelerate the formation of immunosuppressive myeloid cells in the tissue microenvironment of human pancreatic cancer. Cancer Res 2015;75:2629–2640.

87. Chao T, Furth EE, Vonderheide RH. CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. Cancer Immunol Res 2016;4:968–982.

88. Burke SJ, Lu D, Sparer TE, Masi T, Goff MR, Karlstad MD, Collier JJ. NF-kappaB and STAT1 control CXCL1 and CXCL2 gene transcription. Am J Physiol Endocrinol Metab 2014;306:E131–E149.

89. Ijichi H, Chytil A, Gorska AE, Aakre ME, Bierie B, Tada M, Mohri D, Miyabayashi K, Asaoka Y, Maeda S, Ikenoue T, Tateishi K, Wright CV, Koike K, Omata M, Moses HL. Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. J Clin Invest 2011;121:4106–4117.

90. Carstens JL, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, Allison JP, LeBleu VS, Kalluri R. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat Commun 2017;8:15095.

91. Stromnes IM, Hulbert A, Pierce RH, Greenberg PD, Hingorani SR. T-cell localization, activation, and clonal expansion in human pancreatic ductal adenocarcinoma. Cancer Immunol Res 2017;5:978–991.

92. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148:349–361.

93. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 2011;481:85–89.

94. Nielsen SR, Quaranta V, Linford A, Emeagi P, Rainer C, Santos A, Ireland L, Sakai T, Sakai K, Kim YS, Engle D, Campbell F, Palmer D, Ko JH, Tuveson DA, Hirsch E, Mielgo A, Schmid MC. Macrophage-secreted granulin supports pancreatic cancer metastasis by inducing liver fibrosis. Nat Cell Biol 2016;18:549–560.

95. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–867.

96. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005;438:820–827.

97. Lee JW, Stone ML, Porrett PM, Thomas SK, Komar CA, Li JH, Delman D, Graham K, Gladney WL, Hua X, Black TA, Chien AL, Majmundar KS, Thompson JC, Yee SS, O'Hara MH, Aggarwal C, Xin D, Shaked A, Gao M, Liu D, Borad MJ, Ramanathan RK, Carpenter EL, Ji A, de Beer MC, de Beer FC, Webb NR, Beatty GL. Hepatocytes direct the formation of a pro-metastatic niche in the liver. Nature 2019;567:249–252.

98. Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell 2002;2:289–300.

99. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. Nat Cell Biol 2006;8:1369–1375.

100. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, Xiang J, Zhang T, Theilen TM, Garcia-Santos G, Williams C, Ararso Y, Huang Y, Rodrigues G, Shen TL, Labori KJ, Lothe IM, Kure EH, Hernandez J, Doussot A, Ebbesen SH, Grandgenett PM, Hollingsworth MA, Jain M, Mallya K, Batra SK, Jarnagin WR, Schwartz RE, Matei I, Peinado H, Stanger BZ, Bromberg J, Lyden D. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol 2015; 17:816–826.

101. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263–266.

102. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71–78.

103. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grutzmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM, Australian Pancreatic Cancer Genome I, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH,

Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531:47–52.

104. Griesmann H, Drexel C, Milosevic N, Sipos B, Rosendahl J, Gress TM, Michl P. Pharmacological macrophage inhibition decreases metastasis formation in a genetic model of pancreatic cancer. Gut 2017;66:1278–1285.

105. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997;15:2403–2413.

106. Goldstein D, El-Maraghi RH, Hammel P, Heinemann V, Kunzmann V, Sastre J, Scheithauer W, Siena S, Tabernero J, Teixeira L, Tortora G, Van Laethem JL, Young R, Penenberg DN, Lu B, Romano A, Von Hoff DD. nab-Paclitaxel plus gemcitabine for metastatic pancreatic cancer: long-term survival from a phase III trial. J Natl Cancer Inst 2015;107.

107. Halbrook CJ, Pontious C, Kovalenko I, Lapienyte L, Dreyer S, Lee HJ, Thurston G, Zhang Y, Lazarus J, Sajjakulnukit P, Hong HS, Kremer DM, Nelson BS, Kemp S, Zhang L, Chang D, Biankin A, Shi J, Frankel TL, Crawford HC, Morton JP, Pasca di Magliano M, Lyssiotis CA. Macrophage-released pyrimidines inhibit gemcitabine therapy in pancreatic cancer. Cell Metab 2019;29:1390–1399 e6.

108. Nywening TM, Belt BA, Cullinan DR, Panni RZ, Han BJ, Sanford DE, Jacobs RC, Ye J, Patel AA, Gillanders WE, Fields RC, DeNardo DG, Hawkins WG, Goedegebuure P, Linehan DC. Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. Gut 2018;67:1112–1123.

109. Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ, Smith CW. Functional inactivation of neutrophils with a Mac-1 (CD11b/CD18) monoclonal antibody protects against ischemia-reperfusion injury in rat liver. Hepatology 1993;17:915–923.

110. Rogers C, Edelman ER, Simon DI. A mAb to the beta2-leukocyte integrin Mac-1 (CD11b/CD18) reduces intimal thickening after angioplasty or stent implantation in rabbits. Proc Natl Acad Sci U S A 1998;95:10134–10139.

111. Dove A. CD18 trials disappoint again. Nat Biotechnol 2000;18:817–818.

112. Maiguel D, Faridi MH, Wei C, Kuwano Y, Balla KM, Hernandez D, Barth CJ, Lugo G, Donnelly M, Nayer A, Moita LF, Schurer S, Traver D, Ruiz P, Vazquez-Padron RI, Ley K, Reiser J, Gupta V. Small molecule-mediated activation of the integrin CD11b/CD18 reduces inflammatory disease. Sci Signal 2011;4:ra57.

113. Panni RZ, Herndon JM, Zuo C, Hegde S, Hogg GD, Knolhoff BL, Breden MA, Li X, Krisnawan VE, Khan SQ, Schwarz JK, Rogers BE, Fields RC, Hawkins WG, Gupta V, DeNardo DG. Agonism of CD11b reprograms innate immunity to sensitize pancreatic cancer to immunotherapies. Sci Transl Med 2019;11.

114. Zhang Y, Lazarus J, Steele NG, Yan W, Lee HJ, Nwosu ZC, Halbrook CJ, Menjivar RE, Kemp SB, Sirihorachai VR, Velez-Delgado A, Donahue K, Carpenter ES, Brown KL, Irizarry-Negron V, Nevison AC, Vinta A, Anderson MA, Crawford HC, Lyssiotis CA, Frankel TL, Bednar F, Pasca di Magliano M. Regulatory T-cell depletion alters the tumor microenvironment and accelerates pancreatic carcinogenesis. Cancer Discov 2020;10:422–439.

115. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, Yu KH, Hao Y, Wolfgang CL, Park Y, Preall J, Jaffee EM, Califano A, Robson P, Tuveson DA. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov 2019;9:1102–1123.

116. Steele NG, Carpenter ES, Kemp SB, Sirihorachai VR, The S, Delrosario L, Lazarus J, Amir E-aD, Gunchick V, Espinoza C, Bell S, Harris L, Lima F, Irizarry-Negron V, Paglia D, Macchia J, Chu AKY, Schofield H, Wamsteker E-J, Kwon R, Schulman A, Prabhu A, Law R, Sondhi A, Yu J, Patel A, Donahue K, Nathan H, Cho C, Anderson MA, Sahai V, Lyssiotis CA, Zou W, Allen BL, Rao A, Crawford HC, Bednar F, Frankel TL, Pasca di Magliano M. Multimodal mapping of the tumor and peripheral blood immune landscape in human pancreatic cancer. Nature Cancer 2020;1:1097–1112.

117. Schlesinger Y, Yosefov-Levi O, Kolodkin-Gal D, Granit RZ, Peters L, Kalifa R, Xia L, Nasereddin A, Shiff I, Amran O, Nevo Y, Elgavish S, Atlan K, Zamir G, Parnas O. Single-cell transcriptomes of pancreatic preinvasive lesions and cancer reveal acinar metaplastic cells' heterogeneity. Nat Commun 2020;11:4516.

118. Hosein AN, Huang H, Wang Z, Parmar K, Du W, Huang J, Maitra A, Olson E, Verma U, Brekken RA. Cellular heterogeneity during mouse pancreatic ductal adenocarcinoma progression at single-cell resolution. JCI Insight 2019;5.

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Correspondence
Address correspondence to: Howard C. Crawford, PhD, Henry Ford Health System, 2799 West Grand Boulevard, Detroit, Michigan 48202. e-mail: hcrawfo1@hfhs.org; fax: XXX. or Marina Pasca di Magliano. e-mail: marinapa@umich.edu. Q3Q4

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The authors disclose no conflicts. Q5

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