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02 Myeloid Cell Mediated Immune Suppression in Pancreatic 01 Cancer

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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*, *INK4A*).^{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 progression¹⁸ 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; CSF1R, 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 κ B kinase; IL, interleukin; MAPK, mitogen-activated protein kinase; MDSC, myeloid-derived suppressor cell; M-MDSC, mononuclear myeloid-derived suppressor cell; NF- κ B, 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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2352-345X

<https://doi.org/10.1016/j.jcmgh.2021.07.006>

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 anti-tumor 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 activities³⁶ 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

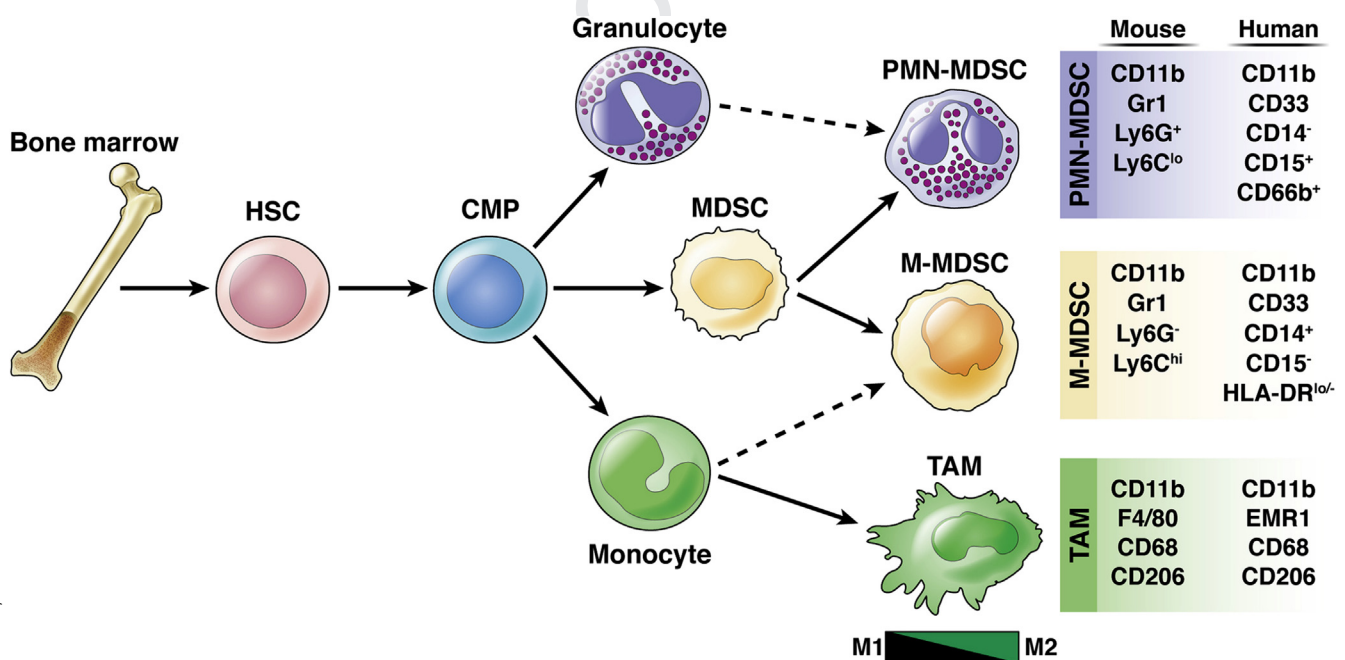


Figure 1. Myeloid cell lineage differentiation and markers. Schematic of myeloid cell differentiation from the bone marrow. Hematopoietic stem cells (HSC) from the bone marrow give rise to common myeloid progenitors (CMP), which give rise to monocytes, granulocytes, and immature myeloid cells, referred 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- κ B) 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 treatment⁷⁶—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

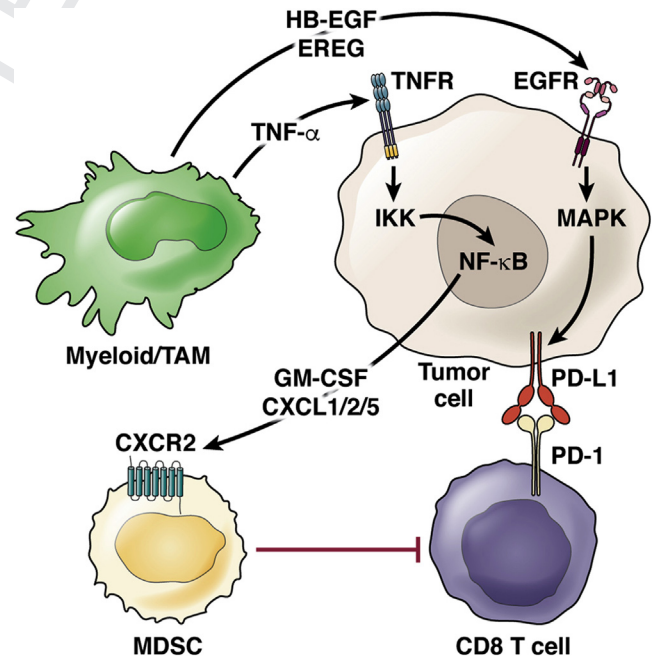


Figure 2. Myeloid-epithelial crosstalk promotes immune suppression. Schematic for cellular crosstalk and corresponding signaling pathways in the PDA TME that contribute to immune suppression. Myeloid cells secrete various ligands, HB-EGF, EREG, and TNF- α , that signal to their respective receptors, EGFR and TNFR, on tumor cells, thus activating EGFR/MAPK and NF- κ B signaling, respectively. MAPK signaling in tumor cells results in elevation of PD-L1 expression, inhibiting CD8⁺ T cells through interaction with PD-1. NF- κ B 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- κ B is a transcription factor with known diverse function in regulation of the immune system.⁸⁰ Dysregulated NF- κ B signaling can lead to inflammatory conditions such as cancer.⁸¹ Along with KRAS, NF- κ B is constitutively active in PDA patients.^{82,83} NF- κ B is held inactive in the cytoplasm in a complex with inhibitory κ B proteins. Extracellular signals, such as TNFR ligation, activate inhibitory κ B kinase (IKK), phosphorylate inhibitory κ B, targeting it for degradation and resulting in the nuclear translocation of NF- κ B complexes to activate transcription of target genes. The IKK complex is made up of 2 kinases, IKK α and IKK β , and an additional subunit, NEMO/IKK γ .⁸⁴ Inactivation of IKK β in PDA tumors reduced infiltration of macrophages and MDSCs and blocked carcinogenesis, extending survival.⁸² Having established that both macrophages and NF- κ B 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- κ B.⁷³ These data suggest NF- κ B is not only critical for PDA formation but also mediates myeloid cell infiltration in the tumor.

NF- κ B 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- κ B 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- κ B signaling in tumor cells, which promotes an immunosuppressive myeloid phenotype, exacerbating disease.

NF- κ B 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 heterogeneous 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 tumor⁹⁵ 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

589 metastatic dissemination. The concept of the pre-metastatic
590 niche is an important question that is relatively unexplored
591 in PDA. Each of these studies provides a framework to
592 explain the role myeloid cells play in pre-metastatic for-
593 mation. Thus, identifying methods to interfere with myeloid
594 function has the potential to mitigate metastasis of this
595 highly aggressive cancer.

596 In addition to their role in tumorigenesis and pre-
597 metastatic niche preparation, myeloid cells have been
598 implicated in migration and invasion of metastatic disease in
599 many cancer types.^{35,101,102} CCR2²⁰ and CXCR2⁶⁸ inhibition
600 reduces metastatic dissemination in PDA through ablation
601 of monocytes/macrophages and MDSCs, respectively. MDSC
602 depletion in mouse PDA tumors converts the tumor from
603 the highly invasive basal subtype to the less aggressive
604 classical subtype and extended survival.^{68,103} Furthermore,
605 pharmacologic depletion of macrophages with liposomal
606 clodronate impairs angiogenesis and reduces metastasis
607 formation in mice with PDA.¹⁰⁴ Myeloid cells appear to be
608 critical for both the formation of the pre-metastatic niche
609 and metastatic dissemination.

611 Macrophages Drive Resistance to 612 Chemotherapy

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

627 Myeloid Cell Compensatory Responses

628 Throughout this review we have highlighted a myriad of
629 reports targeting monocytes/macrophages and MDSCs in
630 PDA. It has become clear that these approaches, while
631 beneficial, often result in a compensatory response of the
632 other myeloid cell subsets. Two studies in PDA report a
633 compensatory increase in monocyte and macrophage sub-
634 sets when MDSCs are depleted.^{71,108} To prevent compen-
635 satory myeloid infiltration, another approach is to target all
636 myeloid cells via integrin CD11b on their surface. Although
637 antagonists for CD11b exist,^{109,110} they have not been well-
638 tolerated in patients because of toxicity.¹¹¹ Instead, an
639 alternative approach to activate CD11b rather than antag-
640 onize has shown promise in preventing inflammation.¹¹²
641 The small molecule CD11b agonist reduces inflammation
642 in a mouse model of PDA.¹¹³ CD11b agonism reduces
643 myeloid infiltration, increases T-cell infiltration, and sensi-
644 tizes tumors to both chemotherapy and immunotherapy.¹¹³
645 Although the total number of myeloid cells was reduced
646 with CD11b agonism, macrophages that remained were
647 reprogrammed, reducing the expression of a number of

648 immunosuppressive genes (expressing Arginase 1, IL10,
649 transforming growth factor beta) and increasing antigen
650 presentation abilities, leading to activation of classical
651 dendritic cells and subsequent T-cell infiltration.¹¹³ CD11b
652 agonism is one potential avenue to avoid myeloid cell
653 compensation when targeting a select myeloid cell subset.

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

667 Myeloid Single Cell Transcriptomics

668 Recent single cell RNA sequencing efforts in PDA have
669 revealed significant heterogeneity within myeloid cell sub-
670 sets that confirm the M1/M2 designation is an over-
671 simplification. Analysis of human PDA tumor samples
672 compared with adjacent normal pancreas tissue identified
673 populations of neutrophils, classical monocytes/macro-
674 phages, resident macrophages, and alternatively activated
675 macrophages.¹¹⁵ *MARCO*, *APOE*, *SPP1*, and *CIQA* emerged as
676 novel macrophage markers that warrant further evaluation
677 in PDA.¹¹⁵ Another study identified similar myeloid pop-
678 ulations in human PDA compared with adjacent normal
679 pancreas tissue with similar gene expression profiles.¹¹⁶
680 Myeloid cells are shown to have heterogenous expression
681 of immune checkpoint receptors (*LGALS9*, *CD274*, *PVR*,
682 *CSF1R*, *SIRPA*, *HLA-DQA1*).¹¹⁶ Putative immune checkpoint
683 interactions were up-regulated in PDA compared with
684 adjacent normal samples, and these interactions were
685 heterogenous across patients.¹¹⁶ Because of the over-
686 whelming lack of response to immunotherapy approaches,
687 these data suggest the heterogeneity of immune checkpoints
688 across patients is a contributing factor, and we should
689 consider the possibility of precision medicine in immuno-
690 modulatory approaches.

691 Two studies used single cell transcriptomics analysis to
692 evaluate the immune response during mouse PDA pro-
693 gression.^{117,118} Consistent with previous reports, macro-
694 phages were identified as one of the major immune cells
695 infiltrating early lesions. Through unbiased clustering, 3
696 macrophage populations were identified in early lesions,
697 whereas only 2 macrophage populations were identified in
698 late/tumor samples.¹¹⁸ The macrophage population only
699 found in early lesion samples had expression of *Fn1*, *Lyz1*,
700 and *Ear1*, suggesting this population is involved in wound
701 repair.¹¹⁸ There was not an equivalent macrophage popu-
702 lation to this one seen in the late-stage tumor samples,
703 suggesting macrophage populations change over the course
704 of disease progression. In a separate study, macrophages
705 from late lesions compared with early lesion samples had an
706 increase in the chemokines, *Cxcl1*, *Cxcl2*, and *Ccl8*, which

707 have known roles in recruitment of MDSCs (*Cxcl1*, *Cxcl2*)
 708 and macrophages (*Ccl8*), suggesting sustained infiltration of
 709 myeloid cells as carcinogenesis progresses.¹¹⁷ These mac-
 710 rophages up-regulated markers of alternative activation
 711 (*Mrc1*), further supporting the concept that macrophage
 712 polarization changes in later stages of PDA. Importantly,
 713 these combined efforts have revealed novel myeloid cells
 714 markers with potential functional importance in PDA.

715 Conclusions and Future Directions

716 In this review we have defined myeloid cell subsets in
 717 the PDA TME and discussed their role in myeloid cell-
 718 mediated immune suppression. We highlight the impor-
 719 tance of myeloid cells through disease progression from
 720 initial formation of ADM to carcinogenesis to the formation
 721 of the pre-metastatic niche leading to ultimate tumor cell
 722 dissemination. Current myeloid targeted approaches in
 723 combination with chemotherapy and immunotherapy regi-
 724 mens relieve this robust immune suppression and activate
 725 T-cell effector responses.

726 However, many questions remain unanswered. The
 727 mechanisms behind the inverse correlation of myeloid cell
 728 and T cells have yet to be fully elucidated. Although we have
 729 some understanding of the pathways involved, we are
 730 lacking the complete picture, especially with respect to the
 731 complex compensatory networks that appear to overcome
 732 monolithic approaches. A better understanding of the
 733 mechanisms behind myeloid-mediated immune suppression
 734 will uncover novel and hopefully targetable components.
 735 With the large influx of single cell transcriptomics data, it
 736 has become even more evident that the M1/M2 designation
 737 is a gross oversimplification and does not accurately mirror
 738 the in vivo heterogeneity of macrophages. These reports
 739 have uncovered novel macrophage markers that may have
 740 functional implications and should be evaluated. Most of the
 741 MDSC work in PDA has targeted the PMN-MDSC subset.
 742 Because the M-MDSCs are more immunosuppressive in
 743 nature, selectively targeting this cell population is of inter-
 744 est. Myeloid cells comprise the largest part of the TME and
 745 are ideal targets to reverse immune suppression.
 746 ⁰⁷

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Received March 10, 2021. Accepted July 14, 2021.

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Conflicts of interest

The authors disclose no conflicts.

Funding

Supported by NIH/NCI grants R01CA151588, R01CA198074 and the American Cancer Society to MPdM. This work was also supported by the NIH U01CA224145 and University of Michigan Cancer Center Support Grant (P30CA046592), including an Administrative Supplement to HCC and MPdM. SBK was supported by NIH T32-GM113900 and NCI F31-CA247076.