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Review

Pancreatic ductal adenocarcinoma immune microenvironment and immunotherapy prospects

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Abstract

The tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) is non-immunogenic, which consists of the stellate cells, fibroblasts, immune cells, extracellular matrix, and some other immune suppressive molecules. This low tumor perfusion microenvironment with physical dense fibrotic stroma shields PDAC from traditional antitumor therapies like chemotherapy and various strategies that have been proven successful in other types of cancer. Immunotherapy has the potential to treat minimal and residual diseases and prevent recurrence with minimal toxicity, and studies in patients with metastatic and nonresectable disease have shown some efficacy. In this review, we highlighted the main components of the pancreatic tumor microenvironment, and meanwhile, summarized the advances of some promising immunotherapies for PDAC, including checkpoint inhibitors, chimeric antigen receptors T cells, and cancer vaccines. Based on our previous researches, we specifically discussed how granulocyte-macrophage colony stimulating factor based pancreatic cancer vaccine prime the pancreatic tumor microenvironment, and introduced some novel immunoadjuvants, like the stimulator of interferon genes.

Keywords: Pancreatic ductal adenocarcinoma; Tumor microenvironment; Immunotherapy; Cancer vaccine; Stimulator of interferon genes

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) has a high mortality rate with the overall 5-year survival of approximately 9%.[1] Tumor immunotherapy is considered to be the most active area of cancer research today, which is designed to improve the immunogenicity of tumor cells, stimulate and enhance the anti-tumor immune response, and finally inhibit tumor growth and progression. Generally, the interactions between the immune system and tumor cells are described as the elimination phase, equilibrium phase, and escape phase. The immune system can rapidly recognize and eliminate transformed cells in the early elimination phase. The escaped tumor cells will then change their own genome and create a tumor microenvironment (TME) suitable for the growth of the early lesions. In the last phase, tumor cells recruit immunosuppressor cells like marrow-derived suppressor cell (MDSC), tumor associated macrophages (TAM), and regulatory T cells (Treg cells), to help establishing an immunosuppressive TME, therefore escaping from host immune surveillance.[2]

As a non-immunogenic tumor, the immune profile of PDAC and immunologic milieu of its TME is unique relative to other malignant tumors that are responsive to immunotherapy. PDAC bears low-moderate mutational burden and has lower immunogenic potential.[3] The TME of PDAC has increased the infiltration of immunosuppressive cells, like MDSCs and Treg cells, and is characterized by increased infiltration of carcinoma associated fibroblasts (CAFs) resulting in collagen deposition with an elevated fibrotic response.[4] The dense stroma of PDAC is composed of extracellular matrix (ECM), pancreatic stellate cells, fibroblasts, myofibroblasts, a variety of immune cells, cytokines, and growth factors, all of which contribute to tumor proliferation and the promotion of metastasis through an intricate interaction.[5] In this review, we discuss the major players in the immune TME of PDAC and highlight the advances of some promising immunotherapeutic strategies used to manage PDAC.

The immune microenvironment of pancreatic cancer

Pancreatic stellate cells

Pancreatic stellate cells (PSCs) mainly distributed around the pancreatic glands that are able to synthesize matrix proteins, matrix metalloproteinases (MMP), and MMP inhibitors that regulate ECM turnover.[6] PSCs can be activated by factors including pro-inflammatory cytokines, oxidant stress, and by factors of particular interest in PDAC such as hypoxia, hyperglycemia, and increased interstitial pressure.[7] The activated PSCs can secrete various growth factors to promote the growth and proliferation of pancreatic cancer cells, inhibit their apoptosis, and enhance their invasion ability.[8,9] PSCs have been confirmed to be the predominant source of collagen in the tumor stroma, and able to secrete ECM proteins like α-smooth muscle actin and collagen. An investigation that specifically decreases myofibroblasts and ECM in PDAC *in vivo* can inhibit tumor growth and enhance the sensitivity of chemotherapy drugs.[10] Some clinical trials targeting ECM and stroma have been investigated, in which hyaluronidase has been considered as a therapeutic breakthrough.[11] A multi-pronged approach aimed at tumor cells as well as stromal elements may be the key to achieve better clinical outcomes in PDAC patients.

Cancer-associated fibroblasts

Activated fibroblasts in the TME are called CAFs and are one of the most predominant cell types found in the stroma with several functional subtypes. Due to the heterogeneity in each subgroup, it is difficult to regulate the TME by targeting fibroblasts.[12] Myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs) are the most common CAFs in PDAC, and a new population of CAFs that defined as antigen-presenting CAFs (apCAFs) was recognized via single-cell RNA sequencing. myCAFs are distributed around acinus and express alpha-SMA, while iCAFs located more distantly from neoplastic cells, which lacked elevated αSMA expression and instead secreted interleukin (IL)-6 and additional inflammatory mediators. apCAFs express MHC class II and CD74, but do not express classical co-stimulatory molecules. They can activate CD4+ T cells in an antigen-specific fashion in a model system, confirming their putative immune-modulatory capacity.[13,14] Interestingly, some CAFs are tumor promotive while some CAFs are tumor inhibitive. For example, iCAFs can secrete ECM and cytokines like IL-6, IL-11, and leukemia inhibitors. These cytokines can activate IL-6R positive malignant cells and myeloid cells to subsequentially activate the STAT-3 signaling pathways to promote tumor growth. In a mouse model, the IL-6R targeted treatment reduced the activation of STAT-3 pathways and enhanced the antitumor response to chemotherapy, suggesting that the IL-6 pathways may be potential therapeutic targets.[15] CAFs can produce tryptophan decomposition enzymes, like indoleamine 2, 3-dioxygenase and arginase, both of which can enhance

the function of immunosuppressive macrophages and inhibit Tregs.[16] In addition, CAFs are reported to significantly increase the release of exosomes when exposed to chemotherapy like gemcitabine, and these exosomes increased chemoresistance-inducing factor in recipient epithelial cells and promote proliferation and drug resistance.[17] However, Ozdemir et al demonstrated that complete depletion of tumor stroma by targeting CAFs accelerated the progression of PDAC with reduced overall survival, underscoring the highly complex nature of tumor stroma. It seems that targeting the pancreatic tumor stroma does not simply require complete ablation, but needs to be carefully modulated.[18]

Myeloid cells

Tumor-reprogrammed myeloid cells not only create a tolerogenic environment by blocking T cell functions and proliferation, but also promote tumor growth by promoting cancer stemness, angiogenesis, stroma deposition, epithelial-to-mesenchymal transition (EMT), and metastasis formation.[19] The most investigated tumor related myeloid cells in PDAC are MDSCs and TAMs. MDSCs are a heterogeneous population of immature bone-marrow-derived cells able to suppress immune responses in TME. Pancreatic cancer cells can induce the mobilization of MDSCs from bone marrow and circulate systematically before being recruited into the TME.[20] With the progressing of the primary tumor, on one hand, pancreatic tumor cells directly produce granulocyte macrophage colony stimulating factor (GM-CSF) to promote MDSCs accumulation in the TME, on the other hand, the hypoxic environment upregulates the secretion of hypoxia-inducible factor 1, which serves as a key mediator for MDSC recruitment.[21,22] The MDSCs in TME suppress T lymphocytes through direct contact and/or through a combination of multiple major mediators such as inducible nitric oxide synthase, cyclooxygenase-2, prostaglandin E2, transforming growth factor (TGF-β), IL-10 and Tregs, et al.[23] What worth mentioning is the interaction between MDSCs and T cells. Laura et al observed that MDSCs are harmless when contacting with resting T cells and become functionally active only in the presence of activated T cells, a crucial interaction capable of inducing a number of events including IL-10 release, STAT3 activation, programmed death protein 1 (PD-1), programmed death-ligand 1 (PD-L1) up-regulation.[24]

TAM differentiation relies on the NOTCH/recombination signal-binding protein for the Ig κ J region signaling pathway and are the major leukocyte population infiltrating cancers.[25] They are attracted into the pancreas by chemoattractants present in the tumor stroma like IL-4 and colony stimulating factor-1 (CSF-1). They can also be generated by a polarization switch from inflammatory M1 macrophages to a tumor-promoting M2-like phenotype.[26] M2 macrophages in tumors is related to early metastasis, tumor recurrence and ultimately reduced overall survival.[27] Targeting chemokine receptor (CCR) 2 positive TAMs alone improves antitumor immunity in preclinical models, and enhanced antitumor immunity and chemotherapeutic responses are observed when in combination with targeting C-X-C chemokine receptor (CXCR) 2 positive tumor associated neutrophils.[28] Genetic or pharmacologic inhibition of phosphoinositide 3-kinase (PI3K) γ, a key macrophage lipid kinase, restores antitumor immune responses and improves responsiveness to standard-of-care chemotherapy in PDAC.[29]

T lymphocytes

The seemingly simple problem of the presence or absence of T cells in pancreatic tumors remains controversial. Although some studies have found CD4+ and CD8+ T cell infiltration in human PDAC biopsies, immunohistochemical analysis suggests that there is also a lack of CD8+ T cells in human tumors. A study of multiplex immunohistochemical analyses for patients with PDAC revealed that CD4+ and CD8+ T cells were present in many tumors, but functional T cells were rarely observed.[30] Another similar study confirmed that PDAC patients with low intratumoral regulatory T cells and high peritumoral CD8+ T cells relate to long-term survival.[31] GVAX, a GM-CSF secreting allogeneic pancreatic cancer vaccine, is able to convert the non-immunogenic PDAC into an immunogenic neoplasm by inducing infiltration of T cells and development of tertiary lymphoid structures in the TME, indicating the potential antitumor effect of T cells in PDAC.[32] The gamma delta (γδ) T cells are also immune functional. A recent study in PDAC showed that γδ T cells were excessively infiltrated in human tumors, and *in vivo* experiments observed suppression of tumor progression by depleting the γδ T cells.[33]

Other types of immune cells

Immune response to tumors in TME is a complicated and multifaceted process which requires the participation of immune cells, cytokines, and microenvironmental elements.[5] Dendritic cells (DC)

maturation is necessary to provide costimulatory signals to T cells, but while DC maturation occurs within tumors, it is often insufficient to induce potent immunity, particularly considering the suppressive mechanisms within PDAC.[34] Some researches have demonstrated that some kind of DC subset both expands Tregs and suppresses CD8 T cells to establish an immunosuppressive microenvironment conducive to metastasis formation, and the tumor-infiltrating Treg cells, in turn, are able to promote immune tolerance by suppressing tumor-associated DC immunogenicity.[35,36] In addition, the mutation of Kras genes promotes the recruitment of B cells, and some kind of B cells, like IL-35-producing B cells, can promote the development of pancreatic neoplasia.[37]

Molecular components

Cytokines within TME are potent immune regulators that generally act in an autocrine or paracrine manner.[38] Due to an imbalance between stimulatory and inhibitory cytokines, PDAC gets accelerated growth. In preclinical PDAC models, exogenous application of IL-12 or interferon-γ (IFNγ) can reprogram myeloid cells in TME, induce T cell responses, and lead to tumor regression.[39] In addition, cytokines can also be immunosuppressive. For example, epidermal growth factor, insulin-like growth factor I, transforming growth factor alpha, IL-1alpha, and IL-6 that secreted by peri-tumoral inflammatory cells or pancreatic cancer itself in an autocrine manner, are able to provide positive signals for pancreatic cancer growth.[40,41] What worth mentioning is the IL-6 given its ability to activate fibroblasts and induce production of ECM.[13] Blocking IL-6 and meanwhile giving chemotherapy in KPC mice can induce tumor cell apoptosis, tumor regressions, and improve overall survival.[42]

ECM and hypovascularity

The chemotherapeutic and radiotherapeutic resistance of PDAC is thought to be mediated mainly by its prominent stroma, which composed of a variety of non-neoplastic cell types and ECM. The deposition of abundant amounts of ECM exerts mechanical as well as biochemical effects on PDAC cells.[4] The extremely complex relationship between the ECM and PDAC has been well-reviewed, and what worth noting is the impairment of tumor perfusion and delivery of anti-tumor drugs resulting from the dense fibrotic stroma.[43] The accumulation of ECM components distorts the normal architecture of pancreatic tissue inducing an abnormal configuration of blood and lymphatic vessels, leading to reduced perfusion and hypoxic condition that ultimately stimulates the PSCs exhibiting highly organized parallel-patterned matrix fibers to promote cancer cell motility.[44,45] Moreover, monocytes and neutrophils effectively infiltrate pancreatic tumors and more than half of them are continually replenished from the circulation.[46] Postcapillary venules, where immune cells can extravasate, tend to be located on the periphery of pancreatic tumors, indicating that myeloid cells are capable of penetrating the TME and more effectively than lymphocytes.[47] Some preclinical studies of ECM-targeted drugs have shown promising effects, and a number of clinical trials are currently investigating agents with the potential to advance the future treatment of PDAC.[48] Alex et al proved that targeting stroma by degrading hyaluronan with pegvorhyaluronidase alfa in combination with GVAX decreased immunosuppressive signaling axis expression and increased expression of tumor-specific IFN-γ, and improved survival.[49] Besides, antiangiogenic therapies have proved not to be a good option for PDAC, and though therapeutic delivery may contribute to these failures, alternative approaches targeting the vasculature remain attractive and potentially feasible.[50]

Immunotherapies for PDAC

Immunotherapy has profoundly impacted cancer treatment, and one of its greatest achievements to date has been how it has propelled advances in cancer research. The novel immunotherapies that manipulate immune checkpoints, viruses that generate an effective immune response, cancer vaccines designed to promote immune stimulation, or T cell therapies engineered for specific antigens have transformed the way to treat cancer (Table 1).[51] Generally, immunotherapies induce anti-tumor responses by reprogramming and augmenting immune surveillance and reducing immune suppression (Fig. 1). Here we highlight some promising immunotherapies for PDAC.

Checkpoint inhibitors

T cell activation occurs under the guidance of the up or down-regulation of both co-stimulatory or co-inhibitory ligands and receptors. Once T cell activation occurs, induction of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) receptors follows, which leads to competitive inhibition of CD28 via the B7-1 and B7-2 ligands, consequently stopping

excessive T cell expansion.[52] As immunosuppressive cells infiltrate and persist in early pre-invasive pancreatic cancer lesions, blocking immunosuppressive signals is essential in enhancing immune-based tumor destruction.[53] Ipilimumab is a fully-humanized monoclonal antibody inhibiting CTLA-4 and was the first checkpoint inhibitor approved by the FDA. In a phase II trial, ipilimumab was administered as a single agent to patients with locally advanced or metastatic pancreatic cancer but did not show efficacy, nor prolong the survival.[54] What encouraging is the clinical trial using ipilimumab in combination with GVAX in previously treated pancreatic cancer, in which both the patients in arm A, ipilimumab single treatment, and patients in arm B, the combination treatments, showed enhanced mesothelin specific CD8+ T cells that correlated with increased survival of >4.3 months, as well as a decline in CA-19.9 levels in some patients compared to ipilimumab alone.[55]

PD-1 and PD-L1 are popular checkpoint inhibitors investigated in PDAC. Like CTLA-4/B7 binding, PD-1/PD-L1 receptor-ligand binding leads to a decrease in T cell proliferation and survival, IFN-γ expression, tumor necrosis factor-α (TNFα), and IL-2 production.[56] However, CTLA-4 affects the T cells at a proximal step in the immune response, while PD-1/PD-L1 impacts T cells in the later stages of the immune response within peripheral tissues.[57] In addition, PD-1 blockade allows dormant T cells to reestablish antitumor T cell activity, whereas CTLA-4 blockade leads to the activation and proliferation of additional T-cell clones, and reduces immunosuppressive Tregs.[58] There is a direct correlation between PD-L1 expression and infiltration of immune cells and the presence of lymphoid aggregates.[59] However, in pancreatic cancer, the expression of PD-L1 is substantially lower on average, reported as low as 12% in untreated patients.[60] Some preclinical researches correlating to focal adhesion kinase (FAK) inhibitors and anti PD-1 antibody are ongoing. FAK is a nonreceptor tyrosine kinase whose activation is correlated with higher fibrosis and poor CD8+ T-cell infiltration. Inhibiting the activation of FAK can reduce intratumoral fibrosis, decrease the accumulation of intratumoral MDSCs, limit tumor progression, and sensitize PDAC to PD-1.[61] Several combination immunotherapy protocols trials are also ongoing (Table 1).[62]

Chimeric antigen receptors (CAR) T cells

Chimeric antigen receptor (CAR) T cell therapy represents a novel therapeutic option for pancreatic cancer. This modality utilizes genetically engineered T cells that are redirected to specific cancer-associated antigens to elicit potent cytotoxic activity.[63] Mesothelin is overexpressed in PDAC compared to its negligible expression in normal pancreas and is correlated with an unfavorable patient outcome.[64] Mesothelin peptide-specific high affinity TCR1045-expressing CD8+ CAR T cells has been introduced and increased overall survival *in vivo*.[65] Besides, mesothelin specific mRNA CAR-T cells were proved to be safe in PDAC patients with minimal off-target effects and infiltrated primary and metastatic sites.[66] In addition, carcinoembryonic antigen, prostate stem cell antigen, and fibroblast activation protein have been introduced to create CAR-T cells for PDAC.[67,68] S Mohammed et al demonstrated the suppressed activity of CAR-T cells in tumor-milieu conditions and the ability of CAR-T cells to thrive in an IL-4-rich microenvironment, resulting in enhanced antitumor activity.[69] Some reports showed that co-administration of checkpoint blocking antibodies might improve CAR-T cell efficacy in PDAC.[70] Understanding the TME, improving the off-target effects of CAR T cell therapy, and incorporation of novel agents in combination with CAR T cell therapy may help accomplish effective treatment outcomes in pancreatic cancer.

Cancer vaccines

Considering PDAC is immune quiescent and naturally resistant to radiation and chemotherapy, alternative treatment options are needed. Cancer vaccines involve administering pancreatic tumor antigens to stimulate the immune system to recognize the distinct, small antigenic differences between tumor cells and normal pancreas cells. These vaccines are developed to exploit and activate both innate and active immune arms to eradicate tumor cells and evade future recurrence of the disease.[71] Currently, there are two major types of therapeutic vaccines in the treatment of pancreatic cancer, whole-cell vaccines, like GVAX, and antigen-specific vaccines, like mesothelin-specific, recombinant live-attenuated, double-deleted Listeria monocytogenes, et al.[32,72]

GVAX is created by transfecting human cytokine GM-CSF genes into human tumor cell lines, where the GM-CSF serves as an immune adjuvant capable of mobilizing monocytes, eosinophils, and lymphocytes into the tumor. It is designed to improve immunogenicity and provide an underlying stimulus for antigen presenting cells. Details about GVAX will be discussed

Table 1
Some Immunological clinical trials currently investigating Pancreatic Cancers.

Clinical Trial Number	Study drugs/Biologicals/Other interventions	Phase
Checkpoint inhibitor		
NCT02648282	Cyclophosphamide, GVAX, Pembrolizumab, SBRT	Phase 2
NCT02305186	Pembrolizumab, Chemoradiation	Phase 1, Phase 2
NCT03264404	Pembrolizumab, Azacitidine	Phase 2
NCT03331562	Pembrolizumab, Paricalcitol	Phase 2
NCT03168139	Olaptesed pegol, Pembrolizumab	Phase 2
NCT03723915	Pembrolizumab, Pelareorep (AN1004)	Phase 2
NCT02930902	Pembrolizumab, Paricalcitol, Gemcitabine, Nab-paclitaxel, Surgical resection	Phase 1
NCT03891979	Pembrolizumab, Ciprofloxacin, Metronidazole	Phase 4
NCT03948763	V941 (mRNA-5671), Pembrolizumab	Phase 1
NCT03184870	BMS-813160, Nivolumab, Leucovorin, Gemcitabine, Nab-paclitaxel, 5-fluorouracil, Irinotecan	Phase 1, Phase 2
NCT02311361	Durvalumab, Tremelimumab, SBRT	Phase 1, Phase 2
NCT03637491	Avelumab, Binimetinib, Talazoparib	Phase 2
NCT03190265	Cyclophosphamide, Nivolumab, Ipilimumab, GVAX, CRS-207	Phase 2
NCT03519308	Nivolumab, Paricalcito, Gemcitabine, Nabpaclitaxel	Early Phase 1
NCT02807844	MCS110, PDR001	Phase 1, Phase 2
NCT03104439	Nivolumab, Ipilimumab, Radiation Therapy	Phase 2
Targeting CAF mediated immunosuppression		
NCT02826486	BL-8040, Pembrolizumab, Onivyde	Phase 2
NCT03277209	Plerixafor	Phase 1
Targeting Myeloid cells		
NCT02345408	CCX872-B, FOLFIRINOX	Phase 1
NCT03767582	SBRT, Nivolumab, BMS-813160, GVAX	Phase 1, Phase 2
NCT03336216	Cabiralizumab, Nivolumab, Oxaliplatin, Irinotecan Hydrochloride, Onivyde, Gemcitabine, Fluorouracil, Nab-paclitaxel, Leucovorin,	Phase 2
Targeting stromal depletion		
NCT04058964	PEGPH20, Pembrolizumab	Phase 2
NCT01959139	PEGPH20, Oxaliplatin, Leucovorin, Irinotecan, 5-fluorouracil	Phase 1, Phase 2
NCT03193190	PEGPH20, Atezolizumab	Phase 1, Phase 2
NCT02715804	PEGPH20, nab-Paclitaxel, Gemcitabine	Phase 3
Chimeric antigen receptors (CAR) T cells		
NCT03497819	CARTmeso CART19	Early Phase 1
NCT03818165	CAR2 Anti-CEA CAR-T cells	Phase 1
NCT04037241	Anti-CEA CAR-T cells, Gemcitabine, nab-Paclitaxel, Onivyde, Adrucil, Leucovorin	Phase 2, Phase 3
NCT03890198	LCAR-C182A cells	Early Phase 1
NCT02744287	BPX-601, Rimiducid	Phase 1, Phase 2
Cancer vaccines (GVAX)		
NCT03153410	Cyclophosphamide, GVAX, Pembrolizumab, IMC-CS4	Early Phase 1
NCT03006302	Epacadostat, Pembrolizumab, Cyclophosphamide, CRS-207, GVAX	
NCT02451982	Cyclophosphamide, GVAX, Nivolumab, Urelumab	Phase 1, Phase 2
NCT03161379	Cyclophosphamide, GVAX, Nivolumab, SBRT	Phase 2
NCT00727441	GVAX, Cyclophosphamide	Phase 2
NCT03153410	Cyclophosphamide, GVAX, Pembrolizumab, IMC-CS4	Early Phase 1
NCT01896869	Ipilimumab, GVAX, FOLFIRINOX	Phase 2
Others		
NCT02907099	CXCR4 Antagonist BL-8040, Pembrolizumab	Phase 2
NCT03727880	Pembrolizumab, Defactinib	Phase 2
NCT02929797	CD8+NKG2D + AKT Cell, Gemcitabine	Early Phase 1
NCT02562898	Ibrutinib, Paclitaxel, Gemcitabine	Phase 1, Phase 2
NCT02923921	Pegilodecakin, FOLFOX	Phase 3
NCT02550327	Nab-paclitaxel, Gemcitabine, Cisplatin, Anakinra	Early Phase 1

Currently active and/or recruiting clinical trials for pancreatic cancers, testing novel drugs alone or in combination with standard of care chemotherapy or other therapies.

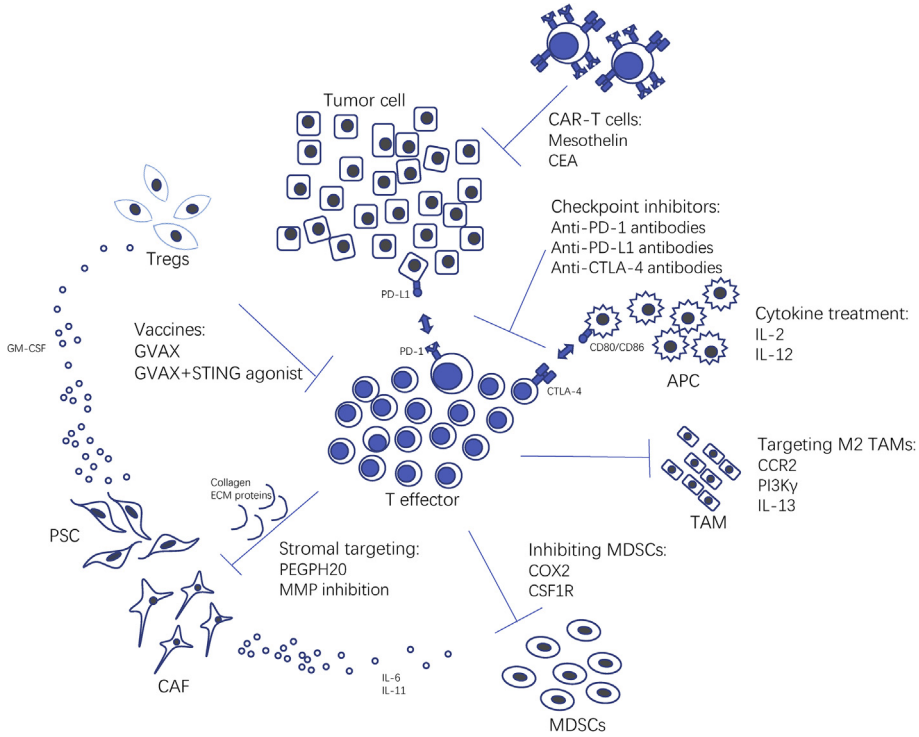

Fig. 1. Immunologic targets within the pancreatic cancer TME. Different cells and molecules within and outside of the TME contribute to effector T cell suppression. Tumor cells express the PD-L1, which can render the effector T cells inhibited and unable to perform effector functions once bound to the PD1 receptor on the surface of T cells. This can be targeted by anti-PD1 or anti-PD-L1 antibodies. Antigen-presenting cells (APC) in the regional lymph nodes expressing CD80/86 on the surface bind to CTLA-4 presenting on the T cells blocking the priming for T cells, which can be specifically targeted by anti-CTLA-4 antibodies. Regulatory T cells (Tregs) are heavily recruited in the pancreatic cancer TME. By applying GVAX alone or in combination with adjuvant like STING agonists lead to reduced Treg recruitment and increased and enhanced effector T cells. T cell suppression can also be inhibited through targeting MDSCs via COX-2 inhibition and targeting the CSF-1 receptor. Targeting tumor-associated macrophages especially the M2 type is another way to regulate suppressive immune microenvironment. Pomalidomide treatment can also reduce M1 to M2 polarization. Targeting the stromal proteins by PEGPH20 and MMP inhibition are promising strategies for better delivery of any therapy including chemotherapeutic drugs. Introduce CAR-T cells, the genetically engineered T cells redirected to specific cancer-associated antigens to elicit potent cytotoxic activity or give exogenous cytokines may also help in treating pancreatic cancer.

in the next chapter. Some novel whole-cell vaccines, like the Listeria-based, Annexin A2-targeting whole-cell vaccine, when introduced in combination with anti-PD-1 antibody, were able to improve PDAC survival outcomes in a pre-clinical murine model and increase the antigen-specific T cell response in the TME.[73]

Antigen-specific vaccines are generally based on tumor antigenic fragments that can induce tumor-specific responses by stimulating T cells.[74] Telomerase tumor antigen is the target for the peptide-based vaccine GV1001, which showed transient and weak Th1-type immune response and reduced infiltration of Treg cells when used with gemcitabine.[75] The Wilms tumor gene 1 (WT1) protein is another suitable vaccine target for PDAC due to its differential overexpression in tumor cells and normal pancreas. WT1-targeted cancer vaccines for patients with PDAC mediated a potent antitumor effect when combined with chemotherapy like gemcitabine in preclinical and clinical studies.[76] Considering that antigen-specific vaccine targeted only one or a few epitopes, whereas PDAC is a poorly immunogenic cancer, a single-agent vaccine that seems to lack significant clinical benefit and durable immunity. The peptide cocktail vaccine displayed a better effect. For example, a phase II clinical trial using OCV-C01, a novel peptide cocktail vaccine that contains epitope peptides derived from KIF20A, vascular endothelial growth factor receptor (VEGFR)1 and VEGFR2 combined with gemcitabine, for surgically resected pancreatic cancer patients. The results proved that OCV-C01 was tolerable with a median disease-free survival of 15.8 months, which was favorable compared with previous data for resected pancreatic cancer.[77]

Immunotherapies targeting TME in PDAC

The barrier of immunotherapies for PDAC is the activated fibroblasts and myeloid cells with immunosuppressive functions within the TME. These cells can be continuously supplemented by local proliferation and the recruitment of monocytes and neutrophils precursors from circulation, and exhibit their immune-suppressive characteristics before T cells respond to their stimuli.[78] In addition, the lack of the effector T cells in the TME, combined with a dense and formidable stroma, and alterations in immune checkpoints all contribute to immunotherapy resistance.[79] Therefore, the TME is an attractive target through which immunotherapy potency and efficacy can be boosted.

Cytokines in PDAC regulate TME, act on cancer cells, and promote tumor evasion. Cytokine treatment can produce an inflammatory response by T cells or induce dendritic cells maturation and proliferation in TME. IL-2, IL-12, and IL-15 as the most potent inducers of anti-tumor activity have been studied among a variety of preclinical researches.[80,81] IL-12 is a natural killer cell stimulatory factor, inducing natural killer cells and activates CD4 positive cells into Th1 type signaling. IL-12 plays an important role in regulating both innate and adaptive immune responses, can by itself induce potent anticancer effects and synergizes with some other cytokines like IL-2, IL-15, IL-21, GM-CSF, TNFα and so on for increased immunoregulatory and antitumor activities.[82] PDAC cells have been found to maintain their metastatic behavior via IL-17B receptor signaling, and treatment with a monoclonal antibody against IL-17RB blocked tumor metastasis and promoted survival in a mouse preclinical xenograft model.[83] TNFα is another promising cytokine that has been proved to play a profound role in malignancy of PDAC, and inhibition of TNFα represents a promising therapeutic option particularly in adjuvant therapy after subtotal pancreatectomy.[84]

Among all cytokines, GM-CSF seems to be the best candidate for adjuvant treatment in immunotherapy. Based on GM-CSF, GVAX can induce the formation of immunologically active tertiary lymphoaggregates, the production of IFN-γ in T effector cells, and the upregulation of the PD-1/PD-L1 pathway in PDAC.[32] Different from primary and secondary lymphoid structures, the formation of tertiary lymphoaggregates depends on antigen stimulation and represents an ongoing adaptive immune response. Evidence supporting their role as regulators includes the expression of early markers of T cell activation, IFNγ, the recruitment of suppressive cell populations, and the upregulation of T cell-suppressing regulatory pathways. However, whether these tertiary lymphoid structures are unique to GVAX treatment or can be elicited by other forms of immunotherapies is not yet known. Interestingly, the immunosuppressive signaling proteins, PD-L1, were present in almost all intratumoral lymphoaggregates, while in untreated patients, or in untreated mice bearing implanted PDAC tumors, PD-L1 was not naturally upregulated.[85] A possible explanations for the upregulation of PD-L1 is the upregulation of IFN-γ produced by the lymphoid aggregate-residing CD4+ and CD8+ T cells.[86] By using multiplex immunohistochemistry and computational image processing analysis techniques to the tertiary lympgoaggregates, our team revealed that differential intratumoral immune complexity stratified therapeutic response to neoadjuvant GVAX in PDAC, and myeloid PD-L1 correlated with activated CD8+ T Cell status.[87] In addition, a suppressed Treg pathway and an enhanced Th17 pathway within these lymphoaggregates were disclosed to be associated with improved survival and increased intratumoral effector T cell to Treg ratios.[32]

Combination therapy with GVAX and PD-1 antibody blockade can improve murine survival compared to PD-1 antibody monotherapy or GVAX therapy alone, and meanwhile, increase effector CD8+ T lymphocytes and tumor-specific IFN-γ production of CD8+ T cells in the TME.[85] To potentiate the activity of GVAX, cyclophosphamide has been incorporated to this combination therapy, which inhibited Tregs and the CTLA-4 expression on T cells, enhanced the antitumor activity induced by vaccination. Clinical trials using GVAX with the PD-1 blockade for PDAC treatment are under processing, and by applying the same techniques on human pre- and post-treatment specimens, we will be able to assess whether anti-PD-1 therapy breaks the balance between T effector, Tregs and different T helper subtypes, and between effector activating and inhibitory immune signals.

The antitumor efficacy of immunotherapies can be enhanced with the utilization of immunoadjuvants. The choice of adjuvants is important because some adjuvants that can stimulate T cell priming have been associated with PDAC development in some preclinical models.[88] Stimulator of interferon genes (STING), is a receptor that is found on a variety of cell types and activates an immune response via tank-binding kinase 1 and interferon regulatory factor 3 in response to cyclic dinucleotides, synthetic cyclic dinucleotides, and bacterial infection.[89,90] STING-deficient mice are unable to generate efficient antitumor T cell responses in melanoma, and the ability of checkpoint inhibitors to

stimulate T cell responses was also abrogated.[91] Activating STING pathway results in cancer cell death in both IFNs-dependent and -independent manner. On one hand, the activation increases the production of IFNs for inducing interferon-stimulated genes to prompt cell death, and on the other hand, it causes cell death via IRF3 interactions to activate the mitochondrial apoptosis.[92] Targeting STING pathway by using STING agonists to produce IFNs to enhance antitumor immune response may provide a promising strategy for immunotherapy.

PancVAX, a neoantigen-targeted vaccine, which was administered together with the STING adjuvant to mice bearing PDAC, activated a neoepitope-specific T cell repertoire within the tumor and caused transient tumor regression. When given in combination with anti-PD-1 and agonist OX40 antibodies, PancVAX showed enhanced and more durable tumor regression and a survival benefit.[93] Combining STING agonist with GVAX, called STINGVAX, enhanced antitumor efficacy and upregulated the expression of PD-L1 in mouse models.[94] Currently, our team is investigating the combinational therapy with STING agonist and anti-PD-1 antibody in a preclinical mouse model of PDAC, and the unpublished data proved the efficacy in increasing the overall survival, which paved the further cocktail vaccination studies using GVAX, the STING agonist, and checkpoint blockade.

However, some studies found that activation of STING pathway may play a dual role in controlling antitumor and pro-tumor immunity. For example, Henrique et al proved that STING agonists can promote tolerogenic responses via STING by activating immunoregulatory mechanisms such as indoleamine 2,3 dioxygenase in Lewis lung carcinoma.[95] Brain cancer cells pass some kind of cyclic dinucleotides to activate STING pathway and thereby resulting astrocyte production of IFNα and tumor necrosis factor to support tumor growth and chemoresistance.[96] There are no similar reports related to PDAC so far, but these studies also remind us of the potential dual effects of STING pathway when using STING agonists as an immunological adjuvant.

Prospective for PDAC

The incidence of PDAC is rising with an over 90% mortality despite the best current treatments accentuates the urgent need to find effective therapies. Currently, the only potential cure for PDAC is surgical resection. However, most patients with local disease recur after surgical resection. Immunotherapy has the potential to treat minimal residual disease and prevent recurrence with minimal toxicity, and studies in patients with metastatic and nonresectable disease have shown some efficacy. Through most studies using immunotherapy as a monotherapy and the widespread implementation of immunotherapy in medical practice, we have begun to identify its shortcomings and the mechanisms behind its resistance. As a result, patient selection based on genetic signatures and expression of checkpoint pathway elements, like PD-L1, has become an active area of research to expand the efficacy and application of immunotherapy. Combination therapies involve combining immunotherapy with other immunotherapy agents for synergy to overcome the resistance of one monotherapy. They also involve combining immunotherapy with traditional therapies like chemotherapy, radiotherapy, tyrosine kinase inhibitors, as well as novel therapies like vaccines, T cell therapy, and TME modulators like the NOD-like receptor family pyrin domain-containing 3 inflammasome.[97]

A deep understanding of the TME and the development of more effectively stimulated T cell response are crucial for the management of pancreatic cancer. Whether it is the immunosuppressive TME, priming of T cells or loss of immunogenicity, these challenges limit the efficacy of immunotherapy. However, they also present the opportunity for novel drug design and novel drug combinations. New advances in the field show that despite the low immunogenicity of the PDAC microenvironment, careful modulation can make pancreatic tumors amenable to immunotherapy.[98] However, PDAC is uniquely characterized by multiple redundant barriers to immunotherapy, and effective therapies will require combination approaches with current agents, many of which are currently still in clinical testing. With continued research, like the introduction of bispecific T-cell engager (BiTE) platform, we can develop unique strategies to better utilize immunotherapy, discover innovative targets for immunotherapy potentiation, more deeply understand the immune-cancer relationship, and, most importantly, improve the outcomes for patients with PDAC.[99]

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Conflict of interest

Lei Zheng receives grant support from Bristol-Meyer Squibb, Merck, iTeos, Amgen, NovaRock, Inxmed, and Halozyme, and received the royalty for licensing GVAX to Aduro Biotech. Lei Zheng is a paid consultant/ Advisory Board Member at Biosion, Alphamab, NovaRock, Akrevia, Sound Biologics, Fusun Biopharmaceutical, Foundation Medicine, Datarevive, and Mingruzhiyao. Lei Zheng holds shares at Alphamab and Mingruzhiyao. SM, MTS, ABB, MZ and DLT have no relevant conflict of interest to report. The other authors declare no conflict of interests.

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